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# PLANT PHYSIOLOGY

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VOLUME 8

1933

WITH SEVEN PLATES AND ONE HUNDRED AND FIFTY FIGURES



SCIENCE PRESS PRINTING CO.  
LANCASTER, PA.

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## ERRATA

### VOLUME 7

Page 606, in formula for value of  $t$ , for " $x_1 - x$ ." read  $\bar{x}_1 - \bar{x}$ .

### VOLUME 8

Page 168, last line, for "Tierschen" read tierischen.

Page 189, fifth line from bottom, for "suelement" read seulement.

Page 199, citation 67, for "psychometric" read psychrometric.

Page 201, citation 105, for "gegenseitigen" read gegenseitigen.

Page 336, table I, last line, for "90" read 133.

# PLANT PHYSIOLOGY

JANUARY, 1933

## SEASONAL ABSORPTION OF NUTRIENT SALTS BY THE FRENCH PRUNE GROWN IN SOLUTION CULTURES

HAROLD L. COLBY

(WITH EIGHT FIGURES)

### Introduction

Splendid theoretical as well as practical work has been done by German scientists on seasonal absorption of nutrient salts from the soil by forest trees, both coniferous and dicotyledonous. As a result, the German foresters have found it desirable to increase the efficiency of the utilization of soil nitrate over the entire season, by employing the mixed type of forest planting, instead of planting pure stands of a single species of timber tree. Different species of forest trees have very different periods of nitrate absorption, ranging from the "early season" nitrate absorbers (the pines, etc.) to the "late fall" absorbers (the horse-chestnut, and others) (32).

No similar seasonal absorption studies have been made for any of our deciduous fruit trees. The practical importance of such studies in relation to commercial fertilization of orchard trees is obvious. The theoretical importance to a final understanding of fruit-tree physiology is perhaps of still greater importance. There is reason to believe that fruit trees obtaining a moderate supply of a given element early in the growing season, but severely starved for that element later in the season, will behave very differently from trees continuously starved, or from trees "fed" only late in the growing season or during the entire season. Seasonal absorption is partly inherent; that is, it is a characteristic of the variety, and partly subject to alterations in the soil solution and to other environmental changes.

### Historical resumé

The course of nutrient absorption throughout the growing season by most of our field-crop plants has been well known for some time. Thorough studies were made on absorption by forage crops, grain crops, and root crops by such German workers as WOLFF (56), KNOP (17), SACHS (41),

LIEBSCHER (21), and others. Yet at the present time, apparently no work has been published on the seasonal absorption in fruit trees. Certainly no such work with trees grown in water culture has previously appeared; yet chemical analyses of particular parts of orchard trees, taken at various seasons of the year, are plentiful enough. The seasonal nutrition of forest trees is comparatively well understood, thanks to the rather recent efforts of the German foresters, KÜBLER (20), BAUER (32), RAMANN (32), GOSSNER (35), SÜCHTING (44), JOHN, DEINES, WEIDELT, and MARSHARD; and of COMBES (8) in France. From RAMANN, who has done most of the chemical work on trees at Munich, comes the following interesting statement: "Die Erkenntnis dass die Nahrstoffaufnahme der Baumarten zeitlich verschieden ist, halte ich für sichergestellt."

It may be well to state here, that while no seasonal absorption studies in fruit trees have been made previously, yet general nutrition studies in citrus have been made by REED and HAAS (36), and in apple, plum, and small fruits by WALLACE (49) and MANN (23).

Since the present experiment deals entirely with water-culture work, it will be profitable to take a brief backward glance over the history of water-culture work with trees, and with field-crop plants. Curiously enough, although so little work has been done with trees growing in solutions, yet water-culture work with plants really began with the growing of *trees* in water! DUHAMEL DU MONCEAU (10) in his early work "Physique des Arbres" (part 2), describes the growth of almond, oak, and chestnut trees in the water of an open fountain in a garden in France, in 1758. The fountain water was from a filtered supply from the River Seine. His oak tree, growing from an acorn accidentally dropped into the fountain, grew for some eight years, with no other mineral nourishment than that supplied by the fountain. At the end of eight years the "tree" was 18 inches high. His almond tree grew well for four years in the same fountain. The chestnut tree apparently grew as well in water as in soil, and was planted in garden soil after two years in the fountain. DUHAMEL was convinced that only water was needed for plant growth; he did not know that his fountain of river-water carried dissolved salts. It is well to note here, that ARISTOTLE also held the idea (perhaps gathered from similar, but unrecorded observations) that water was transformed by living organisms into body tissues. Long afterward, VAN HELMONT thought that his famous willow tree took only water from the soil. DUHAMEL's experiments and his conclusions widely influenced the thought on plant nutrition at the time, and up until 1804, when DE SAUSSURE finally corrected the errors of DUHAMEL's work by growing plants in distilled water with and without added salts. DE SAUSSURE also used single-salt solutions, and demonstrated the differential absorption of salts by plants.

One hundred years later WOLFF and KNOP (57) grew oak trees in water culture (pure salt solutions) to an age of 15 years, probably a long-time record for such work. They placed their trees in river water during each winter, so that exact control of salts supplied to the trees was not attained. At the end of 15 years the oak tree was 1.64 meters high, and the stem circumference was about 5 cm. Fifteen-year-old forest oak trees were 8-9 meters high, with stem circumference of 30.4 cm.

NOBBE (28) had fair success with two-year-old (1 meter high) elm trees in water-culture solutions. He measured the transpiration of these trees and found that it was one-tenth as great during the night as during the day. He also found that "yellow" light apparently checked transpiration more than did "blue" light. NOBBE also found that the ash content of his water-culture trees was usually greater than the ash content of trees grown in soil.

BAIN (1) grew a number of peach and apple seedlings and grape vines in water solutions, studying the sensitivity of the roots to copper in the solution. Apple roots were found most sensitive, peach less so, and grape least sensitive to copper salts. Later REED (36) made use of water cultures in studying the nutrition of young citrus seedlings, etc. Recently COMBES (8) compared the chemical composition of beech trees grown in water cultures with the composition of similar trees grown in forest soil, and obtained some rather interesting results. To date, it must be admitted that in no case have trees grown in water culture made equal growth with trees grown in rich soil. Nor have trees ever been grown to full maturity in water-culture work, something that must be done before tree nutrition studies are placed on a plane with field-crop nutrition studies.

Apparently, then, what knowledge we have of the nutrition of deciduous fruit trees did not come from water-culture work. Instead, small tub sand cultures have been largely used, and orchard fertilizer plot experiments have been carried on in various parts of the world, for many years. Results from the latter type of experimentation may have local importance, but the findings are often misleading, and, because of complicated soil conditions, usually tell us little or nothing of the quantitative needs of the orchard tree. More light has been thrown on tree physiology through sand-culture methods, and through chemical analyses of tree tissue produced in cultures under varying conditions of nutrition, water supply, shading, length of day, etc.

In the field of sand cultures, with deciduous fruit trees, the work of MANN (23) and WALLACE (49, 50) is outstanding. WALLACE interested himself chiefly in the elemental starvation of young trees of apple and plum, and of strawberry and gooseberry plants, etc., among small fruits. He starved particular trees for single elements over periods of three consecutive years, noted differences in growth of roots, stems, and leaves, and

recorded differences in the ash content of various organs after such starvation treatments. He was able to produce characteristic symptoms of phosphorus, magnesium, or potassium starvation, etc., during the first season in which the particular element was withheld from the solution.

WALLACE also studied the combined effects of summer pruning in young trees with starvation for the various elements. However, he made no studies of seasonal absorption by any of his plants or trees.

ROBERTS (39) grew dwarf apple trees in sand cultures with high and low nitrogen, short and long-day conditions, etc., and concluded that the type of growth of a tree is a response to the balance of carbohydrate-nitrogen content, rather than to absolute amounts of these materials present in the tree. Also, trees growing in a high nitrogen culture were capable of storing enough nitrogen in one year to make a normal growth the following year when placed in a nitrogen-free culture.

As to the speed with which deciduous fruit trees can absorb particular ions, transport them to the leaves, and utilize them in synthesis in the leaf or elsewhere, KNOWLTON's (18) work is interesting. He used the "half-tree" nitrogen fertilization method, with bearing apple trees, applying nitrate of soda to the ground under only one side of the tree as the fruit buds were just swelling in spring. Twelve days elapsed before an increase in total nitrogen was noticeable in the fruit buds on the nitrated portion of the tree. Later in the season, quicker response to nitrogen is expected. THOMAS (46) found that a nitrate application to soil on June 6 raised total nitrogen in one-year twigs within one week from the date of fertilization. Foliage color, of course, deepens at about the same rate in response to added nitrate nitrogen.

### Seasonal absorption studies with forest trees

It will be interesting to review briefly the knowledge of seasonal absorption in forest trees. First of all, it may be noted that the ash content of both conifers and dicotyledonous trees varies with the elevation at which the trees are grown, and with the composition of the soil in which they grow. The ash content (in percentage of dry weight) and size of leaves (or needles) decrease very markedly with increased elevation (GRANDEAU 12). However, the course of seasonal absorption may not be particularly different, except at very high altitudes, where a short growing season checks full plant development.

BAUER (5) found that apparently every species of forest tree has a different course of mineral-salt absorption throughout the year, as well as a different curve for dry-weight increase throughout the season. Frequently absorption and dry-weight increase follow closely along the same path, but this is not always the case, and probably never is there perfect correlation

between these two activities. The same tree may have very different seasonal absorption for various ions. Although nitrogen and potassium absorption curves usually run parallel, nitrogen and magnesium, or nitrogen and phosphorus curves may be very different. The fir tree absorbed its nitrogen before June 1 each year, in most cases, with no nitrogen absorption from June 1 to September 15. The horse-chestnut took in its nitrogen from June 1 to November 1, continuing a rapid absorption very late in the fall. Larch trees took in almost no nitrogen in June, but were absorbing potassium, calcium, magnesium, and phosphorus at that time. The maximum absorption rate for nitrogen in the ash tree fell in June; for the beech tree, in August. COMBES (8) reported the maximum absorption of nitrogen in the beech tree in July and in October, although he made no observations in August. According to COMBES, at the time of leaf fall there was a tremendous increase (50 per cent.) in the total nitrogen of the beech tree, not all of the increase being accounted for by the backward movement of nitrogen from the leaves.

Taking the ash tree as somewhat typical of the behavior of dicotyledonous trees, we find that in two-year-old trees (BAUER 4), at the start of the experiment on February 27, the dry weight of the entire root system was equal to 2.2 times the weight of the stems. But by November of the same year (after a season's growth in forest soil) the weight of the stem was almost equal to the weight of the root. As to the absorption of nutrients for the entire tree, for the first period (February 27–May 21) the *dry-weight increase was negligible*, and absorption of potassium, nitrogen, magnesium, etc., was slow; but *the absorption of calcium was rapid*, and actual loss of phosphorus appears to have occurred. Iron absorption was fairly rapid, but reached its maximum rate in the second period (May 21–July 21), then slowed down in the third period (July 21–September 17), and from September 17 to November 17, severe loss (60 per cent.) of iron occurred from the entire plant. The stem lost in total iron in the first period; also in the last period. It must be admitted that iron analyses may not have been entirely accurate, owing partly to the small amounts of the element present in tree tissues.

With reference to nitrogen, both roots and stems lost in absolute total nitrogen in the first period; and the root lost nitrogen again in the second period, in spite of the fact that it had already started gaining in total potassium content, of which it lost 60 per cent. of the total initially present, during the first period. In the first period, also, the root of the ash tree lost magnesium, nitrogen, and phosphorus, and 32 per cent. of its dry weight, but *gained 9 per cent. in total calcium*. During the first period the stem lost only 1 per cent. of its dry weight, far less than that lost by the root. During the second period phosphorus was absorbed rapidly; then the total

phosphorus fell off to the end of the season. In the last period (September 17–November 17), the period of leaf-fall, the tree lost in total calcium, nitrogen, magnesium, iron, phosphorus, and dry weight; but total potassium remained practically constant. BAUER concluded that potassium must be absorbed rather rapidly right to the end of the season.

SÜCHTING (44) ET AL., working with beech and other forest trees, showed that during the winter period (September to January) the tree gained in total phosphorus (very markedly), in total nitrogen, and in total calcium, but lost slightly in potassium. SÜCHTING also compared absorption curves of trees with those of the potato; in both cases the start was made with a rather large storage organ, and in such plants the early spring absorption was *not dominated by potassium absorption*, as is the case in rye, etc., where only small amounts of stored food are present at the beginning of the season, in the seed. The contrast is at least interesting, in view of the possible importance of potassium in carbohydrate synthesis in the plant.

KÜBLER (20) experimented with two plots of young beech trees, one plot being given complete fertilizer and the other plot left unfertilized. In following the seasonal absorption in these two sets of trees, he found that the absorption curves were similar in the early periods (up to July), but from July to September the fertilized trees had far more rapid absorption, and increased in dry weight much more than did the unfertilized trees; in the latter, the dry weight production kept constantly behind the increase in total potassium content. Phosphorus and nitrogen absorption were slower than the dry-weight increase. In the case of fertilized trees, during the July–September period, the dry-weight increase was far more rapid than was the absorption of potassium, or any other element. In the same trees during the period September 17–November 17, the total phosphorus decreased greatly. Yet in the unfertilized trees, phosphorus increased right to the end of the season (November 17).

In the early part of summer, nutrient absorption by these young trees was always far ahead of the dry-weight increase. It is of interest to note that BAUER stated that the *root respiration* of beech, ash, elm, maple, and larch trees in the period March 15 to May 24 was far ahead of the respiration of the tops in every case, thus explaining the great loss of dry weight the young tree undergoes in early spring, most of the loss being caused by root activity. Forest trees appear to have a higher iron content than most of our fruit trees, the roots especially being surprisingly high in this element. KÜBLER for beech trees reported ferric oxide equal to 5 per cent. of the ash in leaves; 4 to 9 per cent. of the ash in stems; and 7 to 13 per cent. of the ash in roots. In his *fertilized* trees, the iron content of *leaves* was about the same as in those just mentioned, but the *iron content of roots and stems* was far lower than was the case in *unfertilized trees*. The fertilizer

that was applied to the trees contained potassium as well as nitrogen and phosphorus. BAUER reported a similar iron content for various organs of fir trees, and a tremendous manganese content, equal to 22 per cent. of the ash in the old needles. KÜBLER, again working with beech trees, found that iron stored in the roots moved into the young shoots, and the root showed a great loss of total iron.

BAUER gives some interesting facts concerning translocation of elements within young (two or three-year-old) ash trees. On May 21, of the total potassium in the leaves, about 46 per cent. had come from old parts of the tree (root or stem), the remainder having been recently absorbed from the soil. Similarly 41 per cent. of the total nitrogen, 50 per cent. of the total magnesium, a small part of the silica, and *100 per cent. of the phosphorus* in these new leaves had come from storage in older tree parts. At this early period, enough calcium had come in from the soil to supply the needs of the leaves.

BAUER then presents his classification of the periodical activities of the ash tree as follows:

- Period 1 (up to May 21).—This period is characterized by a using up of stored mineral and organic matter, with very slight absorption of soil nutrients.
- Period 2 (May 21 to July 9).—Great leaf growth and slow root growth occur in this period.
- Period 3 (July 9 to September 17).—The total-leaf dry weight decreases, and nitrogen, phosphorus, and potassium are lost from the leaves.
- Period 4 (September 17 to November 17).—No great change takes place in either stem or root in spite of the backward movement of materials from the falling leaves.

In the new leaves (of the oak) formed in the spring, 40 per cent. of the nitrogen came from the soil direct. Later in summer, when the second-cycle shoot growth occurred, of the mineral elements moving into the young shoots, the old leaves (of the first cycle) furnished 24 per cent. of all the potassium, *100 per cent. of the calcium*, 52 per cent. of the magnesium, 26 per cent. of the phosphorus, and 62 per cent. of the nitrogen. The total iron content in old leaves decreased at this period, the new leaves obtaining some of their iron supply from the leaves of the first cycle of growth. Incidentally, the rate of mineral and nitrate absorption by oak trees appears to be rather slow throughout the season, as is also their general growth rate.

Finally, then, experiments on forest trees make it clear that here *the most rapid absorption for the season does not always occur at the time of maximum terminal growth of the top*. Where the young tree makes both first and second-cycle top growth, the maximum absorption (in per cent.

of the total for the year) is apt to fall either in the "resting" period between the two cycles of top growth, or in the early part of the second cycle. In young beech trees, in southern Germany, terminal growth is often over by June 1 or before. After a rest of three weeks or so, a second cycle of shoot growth begins. According to BAUER, maximum absorption of nitrogen and potassium in young beech trees occurred in July or early in August, in young ash trees in June, larch trees in August, etc. It is probable that periods of maximum root-length growth coincide with periods of maximum absorption of nitrogen and potassium. Phosphate absorption in forest trees is apt to occur either late in the season, or slowly and evenly throughout the summer and fall. Calcium absorption is usually greatest late in summer, although it is notable that calcium is rather constantly absorbed throughout the season by the broad-leaved forest trees, but not by conifers, apparently.

In rather early spring, calcium alone may be absorbed (as bicarbonate and chloride) by many trees. In the period prior to bursting of the buds, ash trees absorb a certain rather small amount of calcium, potassium, silica, and nitrogen, but no magnesium nor phosphorus. The presence or absence of large quantities of given elements in the soil solution at given times of the year does not usually play a deciding rôle in the time of maximum absorption of these elements by the tree. In fact RAMANN and BAUER suggested "mixed" type planting of woodlots to various tree species, so that utilization of nutrients, nitrogen especially, would be more efficient the year round. Thus fir trees may have completed absorption of nitrogen before the pine trees have even started absorption, etc.

On elemental starvation experiments with forest trees there has been comparatively little work, and most of it deals with conifer trees only. In Germany, VATER (48) and MÖLLER (26) starved a group of pine trees, etc., grown in pot cultures, for magnesium, sulphur, nitrogen, and phosphorus. BURGERSTEIN (6) found that his "minus-nitrogen" pine seedlings showed the usual symptoms of low-nitrogen plants, pale green leaves and stunting of growth, etc. Low-sulphur pines showed very small needles, delicate in texture and of pale yellow color. VATER found his low-phosphate pines grew needles of a bluer tinge than normal plants. MÖLLER found that phosphate or sulphate starvation reduced the development of his pine seedlings more than did magnesium starvation.

In the case of fruit trees, elemental starvation studies have been made recently by WALLACE. At an earlier period MÜLLER-TIURGAU (27) grew young pear trees in low and high nitrogen, phosphorus, potassium, and calcium cultures, respectively, for a period of four years. REMY (37) also grew apples and pears in low nitrogen, potassium, phosphorus, and calcium over a three-year period.

WAGNER (52) reported on 20 years' work in fertilization of trees grown in soil, reporting on pears in low nitrogen, phosphorus, and potassium, as well as on complete fertilizer trials. WAGNER found that after a long period of *potash* starvation, his pear trees gave very poor quality fruit. By other starvation treatments, apparently the quality of the fruit was not greatly affected. Low potassium did not give him an increase in percentage of nitrogen in the leaves, as has been elsewhere reported. Low-nitrogen leaves were very high in phosphate, and contained only 1.23 per cent. nitrogen on a dry-weight basis. The low-phosphate leaves were highest of all in nitrogen (1.69 per cent. dry weight). The complete-fertilizer leaves had 1.5 per cent. nitrogen. The low-phosphate leaves were highest of all in calcium.

REMY found that low nitrogen (under 1.25 per cent. of dry weight) in apple and pear leaves during ripening of the fruit was indicative of insufficient nitrogen for full blossoming in the following year. REMY (38) seems to have grown apple trees in water cultures, starving the trees for phosphorus, in order to determine which organs of the tree showed the effects of low phosphate most markedly. He found that the young vegetative growing points were *not* easily affected by phosphate starvation, but that the older leaves and stem parts were most readily brought to low phosphorus content in the ash.

MÜLLER-THURGAU (27), in his work with pear trees grown for four years in low-potassium cultures, found chlorosis appearing only in the last two years. In his low-nitrogen trees, in the third year only a slight chlorosis appeared; otherwise apparently no leaf troubles were brought on by the treatments. For the entire four years, the poorest yield of fruit was in the lot of low-potassium pear trees. The highest yield came from high-nitrogen trees, followed by high-calcium, high-potassium, and low-phosphate trees. Apparently his soil was well supplied with phosphorus. Nutritional conditions giving high fruit yields did not necessarily seem to give large trunk circumference increases. By the latter measure of growth, low-calcium trees gave the greatest increase, although the fruit yield of the trees was poor. In the fourth year of starvation, the low-potassium trees yielded only one-thirtieth as much as the high-potassium trees.

STEGLICH (43) made ash analyses of entire trees, fruit and all, of apple, pear, plum, and cherry. The series of researches, of which STEGLICH's work was a part, covered a period of 13 years. STEGLICH stated that the yearly nutritional needs for the total growth of one "average" fruit tree in moderate bearing, and 25 cm. in circumference were as tabulated on page 10.

Similar studies were made at a later date by VAN SLYKE, TAYLOR, and ANDREWS (47). STEGLICH also calculated the rate at which the yield of

VARIETY	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	CaO
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1. Apple tree	59	11	51	109
2. Pear tree	37	7	40	69
3. Cherry tree	76	30	95	209
4. Plum tree	34	11	74	75

fruit increased in various species of fruit trees, per cm. increase in trunk circumference, also the total weight of foliage of one tree per cm. circumference. He found that in case of cherry trees, foliage weight was equal to 358 gm. per cm. trunk circumference, while with pear trees the foliage weight was only 105 gm. on the same basis, with apple and plum foliage ranking somewhat above pear foliage. Conduction and transport to the leaves in the cherry must be a more serious problem than in the case of the pear tree. Cherry trees also must be classed as heavy feeders on most of the nutrient salts of the soil.

VARIN SIMON had calculated that a fair sized fruit tree, spreading over 20 square meters of land, produced per year 15 kilos of leaves, 8 kilos of wood, and 100 kilos of fruit. DEGENKOLB, BARTH, and STEGLICH (9) compiled average ash analyses of fruit trees from all over Germany. Of apple, plum, cherry, and pear, the plum leaves ran far higher in nitrogen, phosphorus, and potassium on percentage dry weight than did leaves of the other species.

WAGNER (52) reported on 20 years' work in fertilization of trees grown potassium, magnesium, calcium, and phosphorus respectively, over the rather long period of three years. In his last experiment (1929) he used eighty apple trees on Malling Type Ten standard vegetatively propagated stocks, with twenty trees in each starvation group. Part of his trees were summer-pruned. The leaves and prunings were collected in July, analyzed each year, and the entire trees analyzed at the conclusion of the three-year experiment.

It is well to note that WALLACE's complete culture solution added to the sand was high in K/N ratio, but was otherwise much like the usual type of field-crop culture solution. So little is known of the needs of trees in the way of mineral nutrition that no particular culture solution has ever been worked out for tree cultures. WALLACE reported that growth of the check trees was satisfactory, except for brown spots on the leaves caused by excessively high potassium concentration in the culture. The final results of the three years of starvation did not show entire agreement with his previous results from similar starvation experiments with another variety of apple tree. Usually a minus-potassium treatment results in greatly de-

creased shoot growth, but in WALLACE's late experiments no great checking of terminal growth was observed in such trees. Also, minus-calcium trees gave better shoot growth than did complete-culture trees. But in minus-magnesium cultures, shoot growth was greatly reduced; and by the third year the leaves of these trees were chlorotic, thin in texture, and showed severe breakdown or browning of the tissues. More or less breakdown occurred on leaves of all of his cultures during the later years of the experiment.

Root growth in WALLACE's minus-calcium trees was again reported as being very good. Previously he had reported that low-potassium, low-magnesium, and low-phosphorus trees all produced very poor root systems, more or less injured and blackened. These trees (Cox's Orange Pippin) were also grown in sand cultures. WALLACE believed that his low-potassium tree root systems were too small to absorb water enough for the needs of the top, and that leaf scorch resulted as a consequence.

In his latest work, WALLACE (49) has shown how difficult it is to reduce the potassium, magnesium, or calcium content of leaf or bark tissue in trees by starving them for these particular elements. *After three years* in a minus-potassium culture, leaves of the trees concerned had an ash containing 10.6 per cent.  $K_2O$  on July 7 of the last season, when 20 per cent.  $K_2O$  would perhaps have been ample for normal foliage. His minus-calcium leaves of the same date still had 70 per cent. of normal calcium content; and minus-magnesium leaves still had 40 per cent. of normal magnesium content.

In the summer-pruned series, stems and petioles (prunings of July 7 of *third* year of starvation) showed for the minus-calcium series  $CaO$  equal to 94 per cent. of the normal  $CaO$  content; the minus-potassium series showed 55 per cent. of normal  $MgO$  content.

Concerning the interrelated effects of elemental starvation, minus-potassium tissue was usually high in phosphorus. Calcium starvation did not appreciably raise the potassium content. The potassium content of the complete-culture trees was so high in this case that probably no treatment could have raised it materially without death to the tree. Unfortunately, WALLACE does not include the nitrogen content of his trees in the analyses recorded. As to the total ash content of starved tissue, only magnesium starvation raised the percentage of ash (on a dry-weight basis) in leaves, bark, and wood above the normal content. Potassium or calcium starvation resulted in a lower ash content throughout the tree. Also, the absolute percentage of ash seemed to fluctuate from year to year, within the various groups, just as it does in grain crops, etc.

There are certain things in WALLACE's work that will bear questioning. WALLACE himself states that apparently his calcium-starvation treatment

was not 100 per cent. effective in cutting off the supply of calcium to these trees. The sand used as a culture medium was 99.5 per cent. insoluble in hydrochloric acid. Yet obviously the trees obtained a fairly adequate supply of calcium from the small percentage present as impurity in the sand. Each tree had access to ten liters of sand in the pot. And for three years these trees, though slightly reduced in calcium content, produced normal growth, or better than that of the check trees. No leaf mottling nor chlorosis was reported. It is a curious fact that trees (and annual plants), if partially starved for calcium, fill up their tissues, especially their leaves, with silica. In a sense then, in sand cultures, silica may act as a sparer of calcium in the formation of the middle lamella and elsewhere.

Some time ago, REED and HAAS found that roots of citrus trees, grown in water cultures, were extremely sensitive to the absence of calcium in solution. These roots were able to grow for long periods in a simple calcium chloride solution, with all other elements lacking. They did not grow well in distilled water, unless calcium was added. This may be explained in part by the presence of traces of copper in the solution, but none the less, calcium plays a unique rôle in keeping these roots alive and growing. The growing point is particularly sensitive to the absence of calcium in the culture solution.

After WALLACE's earlier papers, it was thought that perhaps deciduous fruit trees were not at all sensitive to the lack of calcium, and were consequently very different in nutritional requirements from the species of citrus trees used by REED in his experiments. It must be recalled that REED worked with water cultures, while WALLACE's work was with sand cultures; the results are therefore not entirely comparable, the sand furnishing both calcium and silica, and affording better aeration for the roots.

Which type of culture more closely approximates soil conditions? A fine sandy loam soil may well be compared with sand cultures, but a heavy and more or less wet clay loam soil is more likely to provide conditions closely resembling, in many respects, an aerated water culture. Yet in either case there should be little doubt that calcium is necessary for the growth of trees, as well as for all other plants, except possibly the lowest algae and certain fungi. The actual amount of calcium needed by some species of fruit trees may be small.

Sand cultures are very apt to contain fair supplies of calcium and iron, but much less likely to hold adequate supplies of potassium, nitrogen, etc. Consequently potassium and nitrogen-starvation experiments may be carried out in sand cultures to much better advantage than can calcium-starvation tests, as WALLACE's work very clearly shows.

### Experimentation

The purpose of this work was to study the course of potassium and nitrate absorption, and the general nutrition of young two and three-year-old French prune trees throughout the season. These trees were grown in water culture during the course of the experiment; and the culture solution itself was studied in determining the amount and time of absorption of various elements. The water-culture method was used because it allows a comparatively easy and accurate study of absorption, when only a small number of trees is used; also, the identical trees are used throughout the entire season. The alternate method of absorption study is to harvest a certain number of entire trees (roots and tops) periodically throughout the season, analyzing the ash of the whole tree each time. A great many trees must be used in such work, at least 100 trees at each date selected for study; and the time and labor required for digging tree roots from the soil, careful washing, drying, grinding, weighing, ashing the tissues, and analyzing the ash is excessive.

The work was begun in 1928, and carried through the summer of 1930. Some of the trees were grown for three years, others for only two years in water cultures in greenhouses at Berkeley. French prune trees were used throughout the study, with a few apple and pear trees for general observation.

In April, 1928, and March, 1929, one-year-old whips budded on Myrobalan stocks were pruned to a 30-inch head, the roots washed carefully, the entire tree weighed, and set up in jars of aerated culture solution in the greenhouse. The jars used were 4-gallon crocks painted on the inside with black asphaltum. Jar covers were gypsum casts, about one inch thick, the covers being boiled in high-melting-point paraffin and painted with asphaltum. The trees were held in place by fairly loose-fitting corks. Continual aeration was supplied through glass tubes to the bottom center of the jars, which were kept about two-thirds full of solution. The jars were well packed in damp moss on tables about three feet from the ground. The greenhouse temperature was lowered during the day by an electric fan at each end of the greenhouse. Air temperature at the level of the tree foliage was recorded daily at about 11 A. M.

Culture solutions were changed every three or four weeks, or more often if a low-nitrate level made it necessary. Constant checks were kept on the supply of nitrate in the cultures by means of diphenylamine tests. The modified HOAGLAND'S solution used for complete cultures contained the following ion concentrations:  $\text{NO}_3 = 700$  p.p.m.;  $\text{PO}_4 = 10$  p.p.m.;  $\text{SO}_4 = 147$  p.p.m.;  $\text{K} = 150$  p.p.m.;  $\text{Mg} = 37$  p.p.m.;  $\text{Ca} = 150$  p.p.m. Total = 1194 p.p.m.

Foliage color was kept good to excellent in the complete solution cultures by the following iron treatment. Particularly in the early part of

each season, iron was added to each jar frequently, the salts used being both ferric chloride and ferric tartrate. In addition, HOAGLAND'S A-Z mixture was used, the mixture introducing nine other cations and three other anions to the stock solution.

HOPKINS (13) recently found that increasing the amount of citrate in the solution depressed the iron-ion concentration, and that the growth curve of the green alga, *Chlorella*, closely followed the changing concentration of iron ion in the surrounding medium. Within limits, the higher the iron-ion concentration, the greater was the growth of the alga. HOPKINS apparently holds the view that only iron ion is absorbed by plants; more soluble iron would not be available to the plant, unless in the ionized state.

The group of trees intended for starvation included 85 one-year-old prune trees, from which number five were immediately taken out for ash analysis, to serve as a check on the initial condition of the trees, with special reference to the storage of mineral elements and nitrogen. The remaining trees were all set up in the greenhouse, ten trees in each of the groups, as shown in table I. (Average fresh weights are given in each case, for March, 1929, the beginning of the experiment; for December, 1929; and the final weights in September, 1930.)

TABLE I

FRESH WEIGHT INCREASES MADE BY YOUNG FRENCH TREES (STARVATION SERIES)

TREATMENT: CULTURE SOLUTION	AVERAGE INITIAL FRESH WEIGHT MARCH, 1929	AVERAGE FRESH WEIGHT DEC., 1929	GAIN	AVERAGE FRESH WEIGHT SEPT., 1930	GAIN (TOTAL)
	gm.	gm.	gm.	gm.	gm.
Complete	123.0	528.0	405.0	1072.0	949.0
-K	109.4	265.4	156.0	316.0	207.3
-K + Na	132.5	307.5	175.0	386.7	254.2
-Ca*	121.4	206.4	85.0	249.0	127.6
-N	129.8	259.8	130.0	303.0	173.2
-Mg	116.0	294.0	178.0	338.7	222.7
-P	120.2	394.2	274.0	421.0	300.8
-S	120.5	461.5	341.0	673.5	553.0

\* -Ca = actually low Ca solution.

The average initial fresh weight of the entire group was 122 gm. The maximum deviation of a single group from the general mean was approximately 10 per cent.

Also, data from French prune trees grown in the greenhouse the previous year (1928), as well as in 1929, are shown in table II.

TABLE II

FRESH WEIGHT INCREASES MADE BY FRENCH PRUNE TREES OVER A TWO-YEAR PERIOD WITH VARIOUS CULTURE TREATMENTS

GROUP TREATMENT	AVERAGE INITIAL WEIGHT APRIL, 1928	FINAL FRESH WEIGHT DEC., 1929	GAIN
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Ten trees in complete nutrient solution	178.0	946.0	768.0
Ten trees in -K	198.0	434.0	236.0
Five trees in distilled water	164.0	296.0	132.0

### General growth behavior of the trees

It is apparent from table I that the group of trees in minus-calcium solutions made the poorest growth, followed in turn by the trees in minus-nitrate, minus-potassium, minus-magnesium, minus-phosphate, and minus-sulphate solutions. The last-named trees made nearly normal growth, particularly as regards root development; and their total nitrate absorption during the season (1929) was practically equal to that of complete culture trees. On the other hand, minus-calcium trees failed to produce any roots until calcium was added to these cultures for a period of about six weeks, after which they were returned to a minus-calcium solution. The roots of French prune (Myrobalan stock) refused to grow in water culture unless at least a trace of calcium was present in solution. The same situation was found in the case of apple roots (crab stocks), pear roots (French stocks), and peach or almond seedling root stocks. The latter two species were particularly sensitive to a lack of calcium in solution. These facts may appear contradictory to WALLACE's findings for apple roots, but the explanation regarding the sand cultures used by WALLACE has been mentioned.

Figure 1 shows the comparative fresh-weight increase, shoot-length growth, and diameter increase of one-year-old French prune trees during the season of 1929; and figure 2 gives the fresh-weight increase, shoot-length growth, and diameter increase of two-year-old trees during the second year of treatment.

MEVIUS's (24, 25) work on minus-calcium cultures and root growth of various annual plants, as well as the recent work of KOSTYTSCHEW and BERG (19) on the form of calcium present in living tissue, involving the supposition that part of the active calcium is adsorbed by plasma colloids, is full of interest in this regard. Also, WARTHIADI (53), working with wheat plants, found that sand cultures behaved somewhat differently than did water cultures, in which, if the Ca/Mg ratio was 1/10 or 1/20, all the wheat

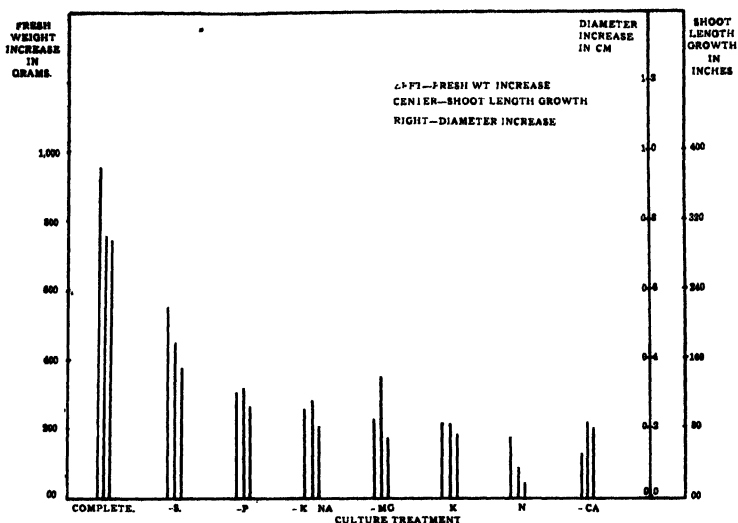


FIG. 1. Average fresh-weight increase, shoot-length growth, and diameter increase per tree. One-year-old French prune trees, season of 1929.

plants died early. In sand cultures, the 1/20 Ca/Mg ratio plants lived, but formed heads containing only chaff. No tillers formed heads in this case. The plants in 1/1 and in 20/1 cultures matured normal heads, in both sand and water cultures.

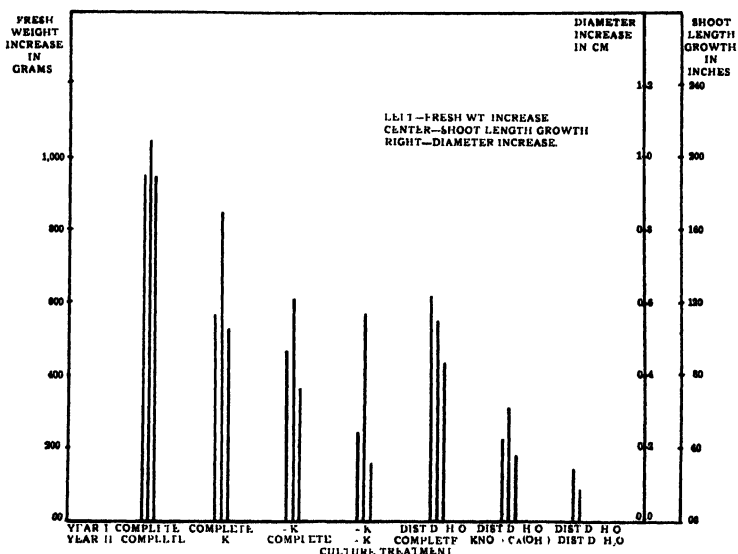


FIG. 2. Average fresh-weight increase, shoot-length growth, and diameter increase made by two-year-old French prune trees in the second year of treatment (1929).

In regard to fruit trees, the white growing roots of French prune trees when placed in a minus-calcium solution soon turned brown or black, and finally died back to the main root. Calcium did not appear to migrate easily in these roots. Portions of a single root system immersed in a plus-calcium solution did not enable the remainder of the root to make a normal growth. The shoot growth of the low-calcium trees was very poor in both years of the experiment. A slight mottling of the leaves on some of the trees was noticeable the second year; otherwise foliage color was good. Each season, however, the foliage was lost rather early (usually in August), the leaves wilting and drying on the tree, apparently suffering from a lack of water.

TABLE III

GROWTH AND ABSORPTION OF ONE AND TWO-YEAR-OLD FRENCH PRUNE TREES  
IN WATER CULTURE

TREATMENT	FRESH WEIGHT INCREASE	DIAMETER INCREASE†	TOTAL NO. ABS'D* IN 1929	TOTAL PO. ABS'D IN 1929
GROUP 1.	gm.	cm.	gm.	gm.
Two-year period				
1928-1929 { Two years in complete solution	765.2	0.96	24.7	0.52
{ Two years in -K solution	235.6	0.155	6.0	0.31
First year in -K; second in complete solution	462.0	0.350	14.4	0.38
First year in complete; second in -K	572.8	0.530	17.5	0.45
First year in distilled H <sub>2</sub> O; second in complete	616.0	0.430	21.7	0.33
Two years in H <sub>2</sub> O	132.8	0.07		
GROUP 2.				
One-year period				
One year in -K solution	156.3	0.110	9.975	0.345
One year in -K + Na solution	177.3	0.147	8.376	0.358
One year in -Ca	85.0	0.062	2.354	0.178
One year in -NO <sub>3</sub>	130.1	0.028		0.190
One year in -Mg	177.5	0.148	7.805	0.275
One year in -PO <sub>4</sub>	274.4	0.149	7.168	
One year in -SO <sub>4</sub>	341.5	0.215	15.808	0.365
One year in complete	405.5	0.324	20.810	0.480

\* Nitrate and phosphate absorption were measured only during 1929.

† Diameter increase is caliper increase of trunk, 3 inches above the bud.

The minus-potassium trees grew moderately well the first year. The second year both shoot and root growth were much reduced, although many white roots were still produced. Potassium-starved roots were in far better condition throughout the two years than were the calcium-starved roots. Leaf scorch and chlorosis appeared in the late summer of the first year, and severe scorch again early in the second summer. During the second season, liberal supplies of iron prevented all but a trace of chlorosis, but did not prevent leaf scorch. It seems that leaves can be kept green, if plants are either in a high-potassium + low-iron solution, or in a low-potassium + high-iron solution. However, after three years of severe potassium starvation, the trees were chlorotic in spite of an abundant iron supply (both injection and solution supply). The diameter growth of the trunks of the minus-potassium trees was greatly checked as the growth tables show (table III). Minus-potassium + sodium trees showed conditions similar to the preceding in every respect. The presence of sodium did not have any effect in preventing leaf scorch or chlorosis.

WIESSMANN (54), working on barley plants, found that  $-K + \text{low } N$  plants matured grain and were nearly normal, while  $-K + \text{high } N$  plants never formed any heads, and produced only two-thirds as great a dry weight as the  $-K + \text{low } N$  plants. Absolute potassium starvation, then, must be studied in the light of relationships with nitrogen supply levels, and probably with other elements as well.

JAMES (15) has reported on work concerning the physiological rôle of potassium in annual plants. He showed that the amount of starch formed per unit leaf area increased with an increase in potassium content. Potassium itself tended to decrease leaf size (unlike calcium), but potassium chloride increased leaf size, the chlorides having the power to increase water content and size of leaves. The apparent rôle of  $KCl$ , in increasing translocation of carbohydrates from the leaves, may possibly be explained on the same basis; *i.e.*, the greater water supply circulating to and from the leaves, caused by the presence of chlorides. Potassium sulphate did not appear to increase translocation of carbohydrates from the leaves.

Trees starved for nitrate showed the usual symptoms of low-nitrogen orchard trees: most severely stunted twig growth, pale yellow-green leaves, a reddish brown bark, and a thin, long, stringy type of root system. The roots continued in active growth throughout most of the entire two years, but terminal top growth lasted only a few days.

The magnesium-starved trees produced very good root growth throughout the two years. The shoot growth was fair the first year, but greatly stunted during the second year, when virtually complete defoliation occurred before July 1 (beginning in May). Severe marginal browning of the leaves, gradually extending back to the midrib, occurred early in both

summers. The symptoms of low-magnesium leaf injury are very typical, the injured portions assuming a dark chocolate color, preceded by olive-green colored areas, water-soaked in appearance. Only a trace of low-magnesium chlorosis appeared the second year, when the iron supply was kept at a high level.

The low-phosphate trees at first made splendid root growth, but later growth was greatly reduced, and very little new root growth was produced during the second year. All roots in the cultures of low phosphate showed darkened tips. Fair shoot growth was made by these trees but was not comparable with normal tree growth; defoliation was exceptionally early in both summers, the earliest of any of the starvation series. In the second summer the leaves were about one-half normal size, and all of them bronzed or turned yellow and abscised in June. The trees made no more new growth after this defoliation. Low-magnesium trees, on the other hand, continually opened up new buds, making feeble shoot growth, only to have that in turn wither and defoliate.

BUTKEWITSCH (7) found that low-phosphate oat plants grew better at pH 5.5 than they did at pH 8.0. The minus-phosphate solution used in the present experiment had a pH of 6.0-6.6. He also notes that low-potassium plants grew better at pH 8.0, quite the opposite situation to that of low-phosphate cultures.

As regards the bronzing (or purpling) of leaves or plants grown in low-phosphate solution, it is suggested that the unusual color development may be the indirect effect of the increased solubility and total supply of iron in the leaf and other active tissues of low-phosphate plants. Soluble-iron compounds are capable of reacting with phenolic, or tannin-like substances, to give various color developments,—brown, purple, red, etc.

The sulphur-starved trees showed excellent root growth in both years, and fair to good shoot growth. Very little premature defoliation occurred in this series, although both brown leaf spots and a pale, light yellow, non-veined chlorosis appeared in both years. Although this sulphur-induced chlorosis is very different in appearance from that produced by low potassium, or by low magnesium, yet in all three cases the development of the typical chlorosis is greatly postponed by high iron supply.

A type of little-leaf can be produced by sulphur starvation treatment, especially if iron is at the same time not above normal. BURGERSTEIN and MÖLLER noted that very small, pale colored needles were produced on young pine trees grown in minus-sulphur cultures. The small leaves produced by low-sulphur French prune trees were usually irregular and lobe-shaped as well as diminutive in size.

The complete-solution trees made good root and shoot growth in both years, and carried well colored foliage. However, the total terminal and

diameter growth was not equal to that produced by well-cared-for orchard trees of the same age in fertile soil in California.

#### SEASONAL ABSORPTION OF NITRATE AND POTASSIUM

During 1929, from May to November, the nitrate absorption of trees of the complete-solution series, as well as those of the various starved groups, was followed, samples being taken from the culture solution every two weeks for nitrate analysis, and for conductivity readings. Nitrate concentration was determined colorimetrically by the phenoldisulphonic acid method. At nitrate levels of 200–300 p.p.m., the error of the method is about 5 per cent., but at low levels of nitrate the error is often more than 10 per cent. The initial level of nitrate in the solutions worked with was 700 p.p.m., and analyses showing levels as low as 5 p.p.m. were regarded as equal to zero, the solution being discarded after checking with diphenylamine and phenoldisulphonic acid. Conductivity measurements were regarded as indicating approximately the course of total salt absorption throughout the season, although it is true that nitrate absorption is revealed by the method, almost to the total exclusion of phosphate absorption, etc.,

TABLE IV

SEASONAL ABSORPTION (BY PERIODS) OF NITRATE BY ONE-YEAR-OLD FRENCH PRUNE TREES (TEN TREES PER GROUP); NO<sub>3</sub> EXPRESSED IN GRAMS

No.	SEASON	SOLUTIONS USED						
		COM- PLETE*	-K	-K+Na	-Ca	-Mg	-PO <sub>4</sub>	-SO <sub>4</sub>
		<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1.	May 17–June 1	3.17	2.856	2.154	No	1.484	-0.336	2.527
2.	June 1–June 15	0.07	1.365	1.656	Root	2.044	3.920	1.575
3.	June 15–June 29	3.22	1.057	0.941	Growth	0.896	0.210	2.191
4.	June 29–July 29†	5.28	4.326	3.585	0.67	3.857	4.109	5.229
5.	July 29–Aug. 10..	0.33	0.483	0.466	0.03	0.693	0.364	0.147
6.	Aug. 10–Aug. 24	0.38	-0.238‡	0.163	0.68	0.07	-1.008	-1.225
7.	Aug. 24–Sept. 6	0.91	0.021	-0.350	0.17	-0.217	0.728	1.274
8.	Sept. 6–Sept. 20	0.89	-0.714	-0.753	0.31	-1.309	-0.819	0.672
9.	Sept. 20–Oct. 5	0.91	0.798	0.552	-0.11	-0.310	-0.728	1.316
10.	Oct. 5–Oct. 19	1.05	0.826	0.428	0.60	0.366	0.042	0.819
11.	Oct. 19–Nov. 2	1.80	-0.665	-0.466		0.371	0.686	1.281
12.	Nov. 2–Nov. 16	0.28	...	...	...	...	...	...
	Total	18.29	9.975	8.376	2.35	7.805	7.168	15.808

\* The complete group here included 15 trees.

† Period of four and one-half weeks.

‡ Minus signs indicate NO<sub>3</sub> lost from the trees back to the culture solution.

and that base exchange and bicarbonate exchange all make conductivity measurements of absorption only approximate.

As has been stated, daily temperature was recorded in the greenhouse throughout both summers (1929 and 1930). A fair correlation between temperature and nitrate absorption (fig. 3) seems to exist. A period of unusually high temperature (with eight consecutive days above 90° F.) and low humidity came at the end of June of the first summer, and the maximum rate of nitrate absorption for the season occurred in this same period. Aside from high temperature, the intensity of sunlight probably reached a maximum at this period also, although no records were kept of it. Top and root growth was fairly active in this period also.

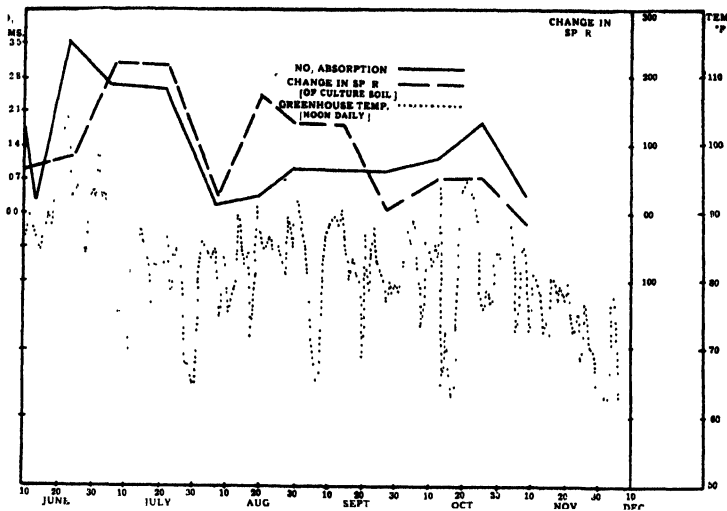


FIG. 3. Rate of nitrate absorption by one-year-old French prune trees in complete culture solution, and the changes in the specific resistance of the culture solutions, plotted with the greenhouse temperature, recorded daily at 11 A. M. Absorption data based on average of 15 trees, during season of 1929. Grams of nitrate per tree per two-week period.

The next high temperature period (fig. 3) fell later in the season, about October 22, with four days of heat above 90° F. At this time root growth and trunk-diameter growth were proceeding rather rapidly, but terminal growth had stopped long before. The second peak in nitrate absorption for the season fell in this period, but was quickly followed by an abrupt decline, as the roots soon went into winter dormancy. Then very slow root growth, or none at all in some cases, was made up to the last days of January, 1930, when vigorous new white feeding roots again appeared, long before the buds started to swell. The trees were placed outdoors in winter.

Between the two peaks of the nitrate absorption curve lay a brief period of very slow absorption, accompanied by low air temperature, followed by a long period characterized by a very uniform rate of nitrate absorption. Long continued periods with temperatures (daytime) below 85° F., probably accompanied by cloudy weather, seemed to be very unfavorable for nitrate absorption in case of the French prune trees. However, very slow nitrate absorption did take place in most of these trees through December, when no temperature data went above 80° F.

The absorption curve presented for 1929 began about June 1. Considerable nitrate absorption occurred in these trees in May, and less in April, as shown by the curve for the same trees for the season of 1930, but the nitrate intake in May was usually below the peak reached in late June. The conductivity curves showed a maximum rate of total absorption in the period July 10–July 30, slightly later than the peak in nitrate absorption.

#### SEASONAL NITRATE ABSORPTION BY TWO-YEAR-OLD FRENCH PRUNE TREES IN 1930

The nitrate absorption of these same trees during their second year of growth in culture solutions was followed, from March 20 to December 1 (table V). Potassium absorption was also followed with the same trees during 1930 (fig. 4). Again it was found that there was a high correlation between daily (noon) temperature in the greenhouse and rate of absorption of both nitrate and potassium. The only marked exception to the correlation came about May 1, midway in the period of rapid terminal shoot

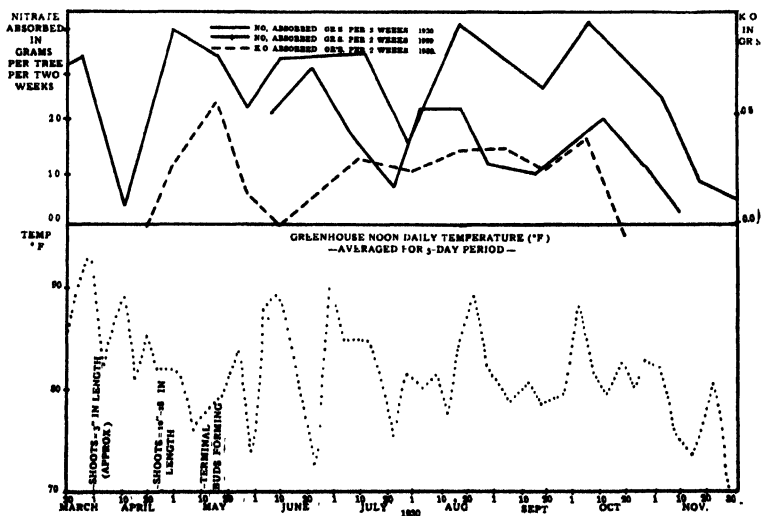


FIG. 4. Rate of nitrate and potassium absorption by two-year-old French prune trees growing in complete culture solution. Average of ten trees in each group.

growth. At this point nitrate and potassium absorption was rapid, in spite of rather low average temperatures. Rapid terminal shoot growth seemed to be an important factor in the rate of nitrate and potassium absorption in these trees. During most of the season, however, temperature appeared to play a dominant rôle.

TABLE V

NITRATE AND POTASSIUM ABSORPTION BY TWO-YEAR-OLD FRENCH PRUNE TREES IN COMPLETE SOLUTION (GRAMS PER TREE); 1930

No.	PERIOD	NO <sub>3</sub> ABSORBED	K <sub>2</sub> O ABSORBED
		<i>gm.</i>	<i>gm.</i>
1	March 15–April 1	3.185	.
2	April 1–April 21*	0.623	–0.044
3	April 21–May 5	3.710	0.240
4	May 5–May 10	3.360	1.080
5	May 19–June 2	2.080	0.280
6	June 2–June 16	3.020	–0.040
7	June 16–July 2	3.560	0.260
8	July 2–July 16	3.180	0.600
9	July 16–August 11*	3.090	0.800
10	Aug. 11–August 25	3.780	0.720
11	Aug. 25–Sept. 8	3.248	0.700
12	Sept. 8–Sept. 29*	3.598	0.780
13	Sept. 29–Oct. 13	3.696	0.840
14	Oct. 13–Oct. 27	2,891	–0.140
15	Oct. 27–Nov. 10	2.303	
16	Nov. 10–Nov. 24	0.644	.
17	Nov. 24–Dec. 16*	0.665	...
	Total	46.9†	5.820

\* Period longer than two weeks.

† N equivalent = 10.5 gm. of total NO<sub>3</sub>.

It may be noted (fig. 4) that the nitrate-absorption curves of two-year trees for 1929 and 1930 agree as to the dates of maximum absorption. The curve for the one-year trees (1929) showed two of the maximum points in common with the curves of absorption by older trees, but showed depressed absorption early in August, in contrast with rapid absorption going on at that time in the case of older trees. One-year trees can hardly be considered typical of usual tree growth or absorption in the orchard, and certainly not in the greenhouse. Trees newly placed in water cultures are late in starting growth, and seldom show second-cycle growth in late summer.

The rate of potassium absorption, it may be noted, usually lags slightly behind that of nitrate absorption, although the rate falls off more abruptly at the end of the season (November 1). Actual exosmosis of K from the

root back to the culture solution takes place at that time. The final drop in the nitrate and potassium absorption curves was correlated closely with leaf fall.

Although regular temperature records were kept only of the air temperature of the greenhouse, some readings taken of the temperature of the culture solution in the jars indicated that the temperature of the solution was being held rather uniformly at low levels, below 60° F., even when the air temperature at mid-day reached 90° F. The jars were well packed in damp moss and were of sufficient capacity to resist marked temperature changes. Any changes of temperature that did occur in the solution of course followed the same trend as that of the air temperature.

Many investigators, dealing with annual plants, have studied the effects of temperature, light, and humidity on the rates of salt absorption. Usually the conclusion has been drawn, from short duration experiments, that humidity and transpiration have no effect on salt absorption other than to increase the percentage of silica or chlorine in the ash of the plants exposed to high transpiration. Some exceptions to this view have been reported. Regarding the effects of light intensity, WIESSMANN (55) reports for rye, barley, and wheat grown in sunlight and in shade, that the former showed the higher total absorption of nitrogen, phosphorus, and potassium. The work of SEIDEN (42) is interesting. Maximum total salt absorption by his plants occurred in the afternoon of each day, at the time of greatest light intensity and highest temperature. Also the percentage of total ash on dry-weight basis increased with rise in temperature of the environment in which the plants were grown. The color of the light to which the plants were exposed also affected the ash content.

Recently PETRIE (30) investigated the effects of temperature on the unequal intake of ions of single-salt solutions by plant tissue. Instead of using entire growing plants, only portions (thin disks) of carrot root were used for the experiments. Such results as he obtained are only indirectly applicable to the problem of salt absorption by entire growing plants, but they appear to be of value, none the less. PETRIE found that at higher temperatures (20° C.) the absorption of cations of single salts is decreased, and the absorption of anions correspondingly increased. The product of the residue of anion and cation remaining in the outer solution remained a constant regardless of temperature. At low temperatures (4° C.) the reverse situation held true (cations being absorbed in excess). Equal rates of absorption for both cations and anions occurred at about 15° C. PETRIE pictures the ions (after absorption) as being held, adsorbed on the surfaces of the negatively charged colloidal particles of protoplasm in the cells of the plant tissue. He proposes that the same process is the basis for selective salt absorption by entire growing plants. Perhaps it is not surprising,

then, that growing fruit trees should show increasing rates of nitrate absorption at higher temperatures.

With forest trees, BAUER and KÜBLER have shown that throughout most of the season, dry-weight production lags well behind nutrient salt absorption, when both are expressed in percentages of the total increase for the entire season. Presumably the same situation holds in fruit trees. Terminal shoot growth may be active in early spring, but the total dry weight of the tree decreases instead of increases at this period. Later in summer, however, there is with forest trees a fair correlation between dry-weight increase and the rate of salt absorption by the roots. It seems fairly certain that nitrate absorption occurs only in growing roots, or at least in white roots, but not in suberized roots. It is doubtful whether any salt absorption (with exception of chlorides) can take place through suberized roots. Experience in the rooting of peach and apple trees seemed to indicate that chloride absorption may occur through old suberized roots in these trees. Leaves and young shoots were forced out much more rapidly on trees with roots in chloride-containing solutions than on trees not receiving chlorides. None of the other salts seemed to affect growth in this way. It should be noted, of course, that dormant trees placed in water solutions always form shoots before any new white absorbing rootlets are formed.

BAUER has discussed the question as to whether or not old fruiting forest trees possess the same or similar seasonal absorption characteristics as the young forest trees that were used in his studies in absorption. He believes that age would make very little difference in the matter, although the oldest trees were four- or five-year-old seedlings. It seems possible that with fruit trees (apple or pear), a heavy crop of fruit might increase the demand for, and absorption of, most nutrients late in the season. At present we have no evidence on the question. Late summer nitrating of apple or pear orchards is usually undesirable, from the standpoints of both fruit color and winter hardiness.

In the case of the French prune, so far as the maximum rate of nitrate absorption is concerned, nitrate fertilization is apparently most needed in late June and early July. The value of nitrate fertilization early in spring, and its effects on fruit setting or on fruit-bud formation, are questions outside our present discussion. A relatively small amount of nitrate added at the right time may be more important in determining the size of the yield than larger amounts of nitrate applied at other times of the season.

It may be noted here that probably not all fruit trees have their maximum nitrate absorption at the same period of the year. Nitrate absorption curves for a few Delicious apple trees (grown in water culture also) showed in early summer a slowly rising nitrate absorption with a small peak about June 25, corresponding to the nitrate maximum rate in the French prune;

but the second peak, the maximum rate of absorption for the whole season, fell on September 2, and the final smaller peak on November 10. The leaves of these trees were green until December. Second-cycle terminal shoots were still growing on September 2.

#### NITRATE ABSORPTION BY STARVED TREES (ONE YEAR OLD)

As has been stated, the seasonal absorption of nitrate by the French prune trees was followed through 1929, for the whole series of starved trees, as well as for those trees in complete cultures. The amount of nitrate and phosphate absorbed for the season was determined for trees of each starvation group, as well as the tree-weight increase, the diameter increase, the total length of shoot growth, etc., during the year of the study.

The set of curves of total nitrate absorption of trees starved for various elements presents a rather interesting situation (figs. 5, 6). Trees starved

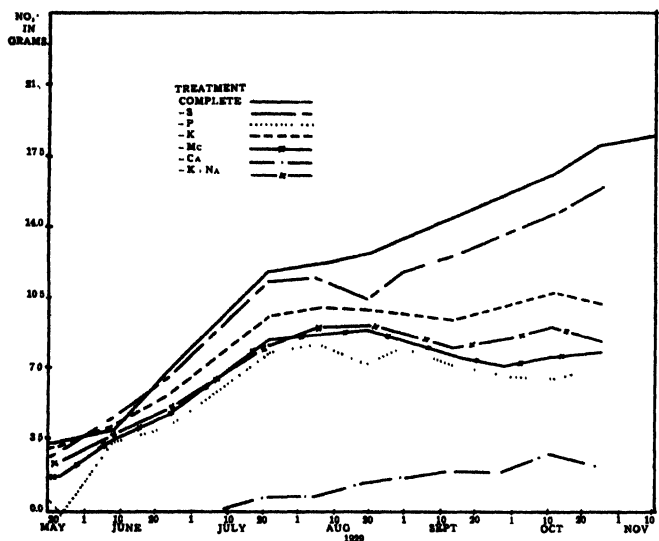


FIG. 5. Total nitrate absorption per tree for season of 1929. One-year-old French prune trees in various solutions; ten trees per group.

for sulphur throughout the year show both a rate and total amount of nitrate absorption very nearly equal to that of complete-solution trees. In fact, early in July the -S trees showed a higher rate of nitrate intake than did the complete, or any other treatment group of trees. This fact might be anticipated, if we consider that  $\text{SO}_4$  is a competitor of  $\text{NO}_3$  in total salt absorption, and if we assume that the -S trees are not very greatly depleted in sulphur at this early time in the season, an assumption which is probably true, since the tree growth was nearly normal until midsummer.

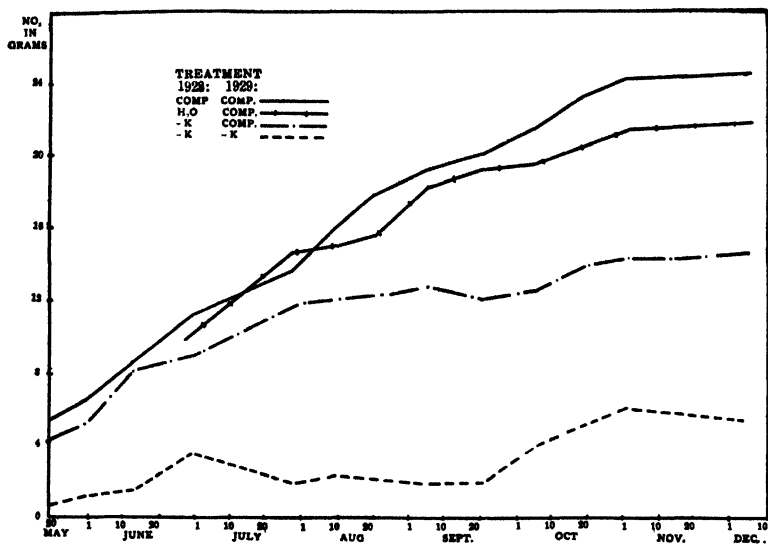


FIG. 6. Total nitrate absorption during season of 1929, by two-year-old French prune trees growing in various solutions.

Following the --S trees, the group in --K showed the next highest rate (and total) for nitrate absorption; it was nearly equal to that of the --S trees up to July 30, when the absorption of the --K trees fell off badly. In fact, loss of nitrate from the trees (by exosmosis into the culture solution) occurred before the close of the season. It should be noted that no loss of nitrate from trees in complete cultures occurred at any time of the season from March to December 1, and only slight losses during the winter months.

In spite of the fact that the group of trees in --K + Na made greater growth than those in --K, the nitrate absorption of the former was far less than that of the --K trees. The presence of sodium may actually be detrimental to nitrate absorption. During much of the season the --K + Na group showed a slower rate of nitrate intake than any other group of trees except the low-calcium group.

Groups of trees in --Mg and in --P differed only little from those in --K + Na in their nitrate absorption, all being very poor. This was true in spite of the fact that the --Mg trees made a terminal growth nearly equal to that made by the --S trees, although the fresh-weight increase and increase in trunk diameter (3 inches above the bud) were very much reduced in the case of --Mg trees. The --S trees were comparatively poor in trunk-diameter increase, making only one-half that made by complete-solution trees, although the total fresh-weight increase of the --S trees was equal to 85 per cent. of that made by the check trees. In short, the --S trees grew

unusually fine root systems, but top growth was not proportionally as good as root growth.

The absence of  $\text{PO}_4$  did not diminish weight increase nearly as greatly as did magnesium starvation. The foliage of the  $-\text{P}$  trees during the first year (1929) was badly injured. The least gain in fresh weight occurred in the  $-\text{Ca}$  group of trees. The  $-\text{NO}_3$  trees, in spite of very poor terminal growth, make proportionately very good weight increases, although both gains are small compared with the gains of complete-solution trees.

A group of French prune trees one year older than the preceding series was completely starved for two years, growing in distilled water; another group was grown in complete solution; a third group was grown in pure water the first year, and placed in complete solution the second year. Completely starved trees showed at the end of two years actual loss in trunk diameter, although considerable shoot growth had been made from reserves in the trees and the average fresh-weight increase per tree for the two years was about 160 gm., the initial fresh weight of the trees being on the average 164 gm.

Trees starved completely for one year, and then transplanted to complete solutions the second year, showed that terminal growth and total weight increase are made somewhat at the expense of diameter increase, which still lags notably behind for a year or so, until the tree has caught up with its salt absorption.

Also in the block of older French prune trees, to which reference has been made, was a group of trees starved for potassium over a two-year period. On April 12 of the third year of starvation, some of these trees were transferred to a  $+\text{K}$  solution, and developed excellent shoot growth and fine leaf color in about two weeks' time. Only a trace of the former chlorosis remained near the midrib portions of the leaf blade. The leaf color had markedly improved 5 days after the potassium was added to the solution. Later in the summer, the tip leaves of the young shoots of these same trees showed excessive reddening, then browning or burning and drying up. The shoot tip itself died back 6–8 inches.

Other French prune trees, also previously starved for potassium for two years, and then placed in complete-solution cultures on May 15, or on June 25 of the third year, showed no shoot growth response whatever to the added potassium. Leaf color did change in about ten to twelve days, and the live parts of badly scorched, chlorotic leaves turned green except for small areas bordering on the scorched margins of the leaf blades.

The roots of all of these trees responded quickly to added potassium, making abundant root growth. Yet it appears impossible to stimulate shoot growth in these trees by adding potassium after about May 1.

## CONDUCTIVITY MEASUREMENTS

The curves of specific resistance (figs. 7, 8) of the solutions of the various starvation groups are worthy of notice. Perhaps the most striking observation was that nitrate-starved trees, in spite of moderate, healthy root growth, were apparently losing certain nutrient salts to the external

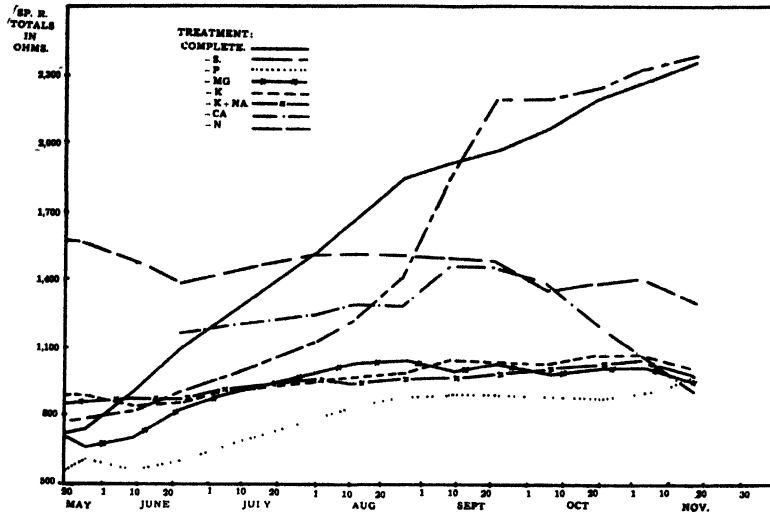


FIG. 7. Total change in specific resistance of culture solutions during season of 1929.

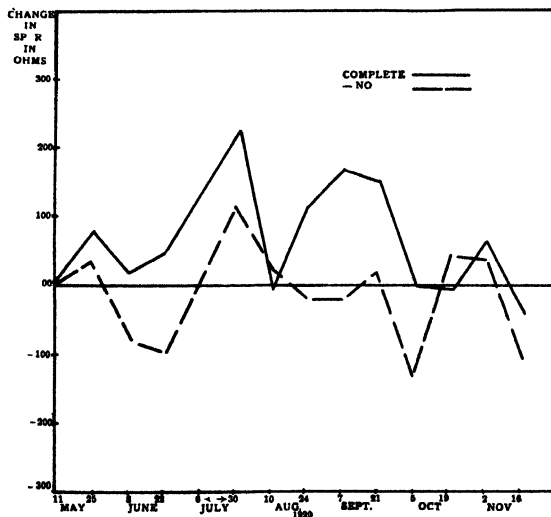


FIG. 8. Rise and fall of the conductivity of the culture solutions (change in conductivity in ohms, per two-week period). One-year-old French prune trees, season of 1929.

solution, throughout most of the season. The low-calcium trees, after calcium starvation became severe (these trees were fed calcium during the early summer to start root growth), appeared to lose an amount of nutrient salts nearly equal to the total previously absorbed in the same season. As mentioned, however, nitrate (perhaps already assimilated) was not the ion lost back to the solution in this period of exosmotic migration. Presumably sulphates, chlorides, phosphates, and bicarbonates were excreted or diffused back into the solution from dying rootlets. There seems to be no doubt that fruit tree roots starved for calcium made very poor nutrient absorbing systems. Apparently their usual properties of semipermeability are lost in such circumstances, and browning and rotting of the young root tips soon follow the change in permeability. On the other hand, it has been found that a solution of calcium hydrate alone, at pH 7.2-7.4, provides a very good medium for healthy root growth by fruit trees. As WOLFF showed long ago, young forest trees can be grown for long periods in calcium chloride solutions alone. HEILBRUNN'S work on calcium and membrane "healing," permeability, protoplasmic vacuolation and streaming in amoeba, etc., is all pertinent to the problem, as is also FARR'S (11) recent work. FARR has shown that for root-hair growth by such plants as the collard, *Brassica oleracea*, only calcium need be present in the external bathing solution. Calcium hydrate solution at pH 10 gave the fastest root-hair growth of any calcium salt used; and the maximum rate of root growth occurred at pH 8.0-8.5 in short-period experiments.

In the present work it has been found that it is entirely possible to transfer fruit trees in mid-season, from complete solution to simple  $\text{Ca}(\text{OH})_2$  solution of pH 7.2 or *vice versa*, without any apparent injury to the young white roots, or to older roots, and without any exosmosis of chlorides taking place. Outward diffusion of the latter usually occurs if the permeability of the tissues containing chlorides is appreciably altered from the normal. Such mid-season transfers as have been described make possible a new type of seasonal absorption work; the trees may be grown in complete solution for one, two, or three months, and then completely starved except for calcium during the rest of the year. Growth observations on such trees should show how dependent (or independent) shoot growth is on recently absorbed nutrient salts.

### Summary

1. A review of the literature of water-culture studies with trees is presented, extending from the time of DUHAMEL DU MONCEAU in 1755 down to the present date.

2. The course of seasonal nitrate absorption, and of elemental starvation, was studied in young one and two-year-old French prune trees, as grown in water cultures in the greenhouse.

3. The effects of elemental starvation (for elements other than nitrogen) on seasonal and total-nitrate absorption are shown for trees of the French prune variety. Sulphate starvation appeared to have a far less depressing effect on nitrate absorption than did starvation for any other of the six major elements of the complete-culture solution. (Iron starvation was not studied in this series.) Potassium, magnesium, and phosphorus starvation all very seriously depressed nitrate absorption, even resulting in loss of nitrate from the roots, late in the season. Calcium starvation prevented root growth entirely. Low-calcium trees (fed a small amount of calcium) absorbed very little nitrate, and lost solutes from the roots when placed in a minus-calcium solution. The root tips of these trees invariably turned brown and died. Roots previously well stocked with calcium survived somewhat longer than the low-calcium roots when both were placed in minus-calcium solutions.

4. The total phosphate absorption for the season was far more badly depressed by magnesium starvation than by potassium or sulphur starvation. Calcium starvation apparently prevented absorption of any considerable quantity of any ion, including phosphate.

5. The primary peak in the seasonal nitrate absorption curve of the complete-solution French prune trees (both one and two-year-old groups) occurred near the end of June, in 1929; with a final, secondary peak falling in late October, followed by a rapid decline to winter dormancy. The complete-solution trees, in 1930, now two years old, again gave the maximum monthly total nitrate absorption in the period June 10–July 10, with brief periods of rapid nitrate absorption coming about May 1, August 20, and October 7. The curve for potassium absorption follows closely that for nitrate absorption, lagging slightly behind in the earlier part of the season.

6. There is a high correlation between temperature (probably light intensity also) and the rate of nitrate and potassium absorption by these trees.

The kind assistance of Dr. J. P. BENNETT, under whose direction this problem was carried out, is gratefully acknowledged.

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# PHYSIOLOGICAL ANATOMY, TYPE, VARIETY, AND MATURITY OF CITRUS FRUITS AS AFFECTING QUALITY OF PREPARED JUICES

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AND ARTHUR L. STAHL

(WITH ONE FIGURE)

## Introduction

The anatomy of different citrus fruits varies with the type, the variety, the quality, and the degree of maturity of the material used, and these factors are of great importance in any consideration of the problems encountered in the preparation of citrus juices. It is of first importance to know how the anatomical structure and the physiological state of the material affect the development of undesirable tastes, changes in the color, and the settling of suspended particles in prepared juices on aging.

The preliminary results presented in this paper concern the localization in the complex tissues of the fruit of the causal agent or agents for changes observed in the prepared juice, and the effect of the citrus type, variety, and maturity factors on the physiological anatomy of the fruit used.

## Plan of experiment

The causes of any undesirable changes in prepared citrus juices from the time of preparation until used may be classified under two heads: (a) those having their origin in the prepared juice itself as a result of the amounts and character of solutes and extractives released by the method of preparation; (b) those present in the external environment, such as gases (particularly oxygen), containers, temperature, and methods of storage. Such factors could operate independently or in combination.

A detailed description of the citrus fruit type is given by FAWCETT and LEE (15). In addition to the tissues mentioned by these authorities, the seeds should be indicated which are attached by means of placentae to the locular walls where these come in contact with the central axis of the fruit (fig. 1). It is also of interest to note that among the constituents of certain of the tissues various glucosides occur (20, 24, 21, 46), and it has been shown (46) that the glucoside content decreases with maturity. Pectic substances are also known to occur in citrus fruits.

Throughout its development the citrus fruit is continually changing in composition and, as a consequence, the extracted juice varies from sample to sample. In the whole fruit the juice is contained within the juice sacs, or vesicles, and does not come into contact with the various tissues that make up the remainder of the fruit structure. In processes of juice extraction, however, the various tissues are broken down and the juice mixed with

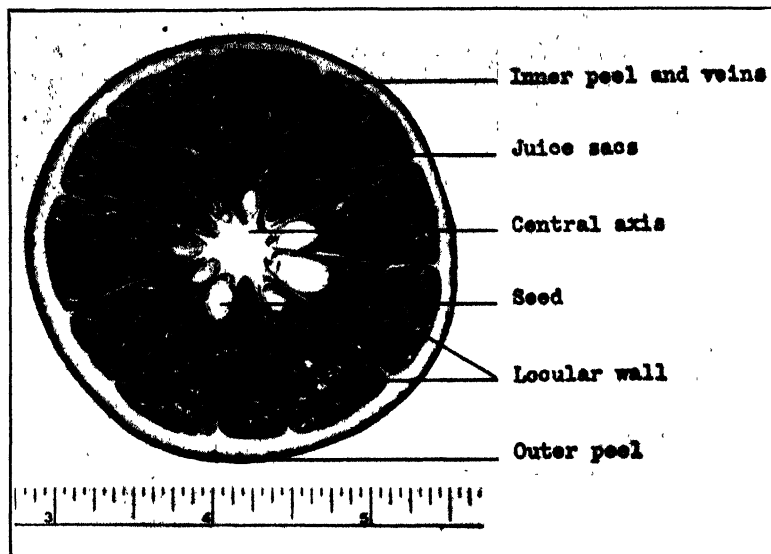


FIG. 1. Cross-sectional view of Parson Brown orange showing tissues.

them. Moreover, the extracted juice contains more or less of these macerated tissues in the form of solid particles and can take up from them any substances that they may contain.

Citrus juice extracted by the usual methods is a buffered solution with an effective acidity, according to reported experiments, ranging from pH 2.1 to pH 4.3. The more apparent solutes are total acids, 0.25 to 11 per cent.; total sugars, 1.5 to 16 per cent.; protein, 0.40 to 1.00 per cent.; and mineral salts, 0.20 to 0.40 per cent. (38, 37, 28, 32, 33, 5, 10, 17). In addition there are undoubtedly certain original solutes whose character has not been determined, as well as extracted solutes and suspended particles which originate mainly in the fruit tissues outside the juice sacs (1, 3, 16). These extracted solutes may include, among others, pectin, glucosides, aldehydes, and esters; and the suspended particles may consist of plastid pigments, citrus oil (in some methods of preparation), and macerated and torn tissue fragments of different sizes derived from the various tissues of the fruit. Extracted citrus juice apparently has a colloidal nature and as such shows marked individuality from sample to sample. Like most fluids of biological origin, the juice from citrus fruits is unstable or constantly changing in composition, but after extraction it has a tendency to become stable.

### Materials and methods

#### PHYSIOLOGICAL ANATOMY

The fruit was separated into the component parts of its various tissues (36). The effect of these constituents when added to the relatively pure

juice was then determined. It was impracticable to separate single tissues, of course, and in carrying on the work groups of allied tissues were considered, such as locular walls, albedo (the inner peel), or outer peel, as shown in figure 1. In the discussion therefore each of these groups is referred to as a tissue. Eight such tissues were considered:

1. Outer peel (epidermal layer with some inner peel attached)
2. Inner peel and veins (albedo)
3. Locular walls
4. Central axis
5. Seed coat
6. Cotyledons and germ
7. Empty juice sacs
8. Juice expressed from filled juice sacs only

Infusions were made by grinding separately with sharp clean sand in a mortar equal portions by weight of the various tissues as listed. These infusions were then made up to volume and used as indicated later in this section. Certain of the constituents of the tissues were studied, particularly the oil, the oil-free sap from the outer peel, and the glucosides from the inner peel and veins.

#### TYPE, VARIETY, AND SOURCE OF FRUIT

The fruits used were grown in various parts of Florida. Satsuma oranges were obtained from Glen St. Mary, Green Cove Springs, Pensacola, Marianna, and Gainesville; Parson Brown from Lake Alfred and Lady Lake; Hamlin from Grand Island; Washington Navel from Gainesville; and the Pineapple variety from Lake Alfred. Tangerines were obtained from Bartow, Hawthorne, and the Experiment Station at Gainesville; grapefruit from Lake Alfred and Gainesville; Key limes from Islamorada; Tahiti limes from Loughman, and a variant of the Tahiti lime from Homestead.

#### MATURITY OF FRUIT

Owing to the fact that the work herein reported was carried out during a comparatively short period early in the fruit season, it was impossible to obtain much information on maturity; however, every effort was made to select fruit in such a way as to cover this subject as thoroughly as possible. The samples of Satsuma fruits varied from under-ripe to over-ripe, and to a lesser extent the same was true of the Parson Brown. The Pineapple and Navel oranges and the tangerines used in most of the experiments were a little below full maturity. Since the variety factor is of extreme importance, as many varieties as possible were studied and compared.

#### METHODS OF PREPARING JUICES

Descriptions of the various methods of preparing citrus juices dealing chiefly with the methods of bottling and sterilization of the juice after sedi-

mentation and clarification (13, 12, 7, 4) or of carbonating the product (18) appear in the literature. The methods of preparing citrus juices to be preserved by the newer "frozen pack" (2) development as considered in this paper on the basis of the physiological anatomy of the material used may be characterized as follows:

A. Relatively small amounts of tissue introduced:

(1) Little peel oil introduced

Method no. 1

Method no. 1a

(2) More peel oil introduced

Method no. 2

B. Relatively greater amounts of tissue introduced:

(1) By grinding, but no oil

Method no. 3

(2) By reaming, and very small amounts of oil

Method no. 4

**METHOD NO. 1.**—This was devised as a control method, in that it provided juice that was virtually free from all tissues except juice-sac constituents. To obtain the juice sacs, the outer rind containing the oil glands was first removed by peeling; the outer wall of the segments with all attached inner peel tissue was then pared off with a stainless steel knife; finally the segments were separated and the segment walls removed by inserting the knife blade at the base of the segment along the central axis and sliding it outward along the locular wall, thus separating it from the juice-sac tissues. The collected tissue was wrapped in clean gauze and pressed between porcelain plates. No metal except the blades of the knives used in paring and dissecting came in contact with the juice in this method.

**METHOD NO. 1A.**—A variation of the first method was used to some extent in studying the normal physiology of citrus fruits. The locules (segments) of the peeled halved fruits were pressed between porcelain plates and the pressed juice collected. Since the juice did not remain in contact with either the locular walls or the inner peel and veins for any great length of time when pressed only once, there was little possibility of the leaching out of constituents from the locular walls or any adhering tissue.

**METHOD NO. 2.**—The juice was pressed from the halved fruits by an inverted aluminum cup which held the fruit and pressed it against a metal form having the same shape as the cup. The clearance between the form and the cup was adjusted to a little more than the thickness of the peel. The shape of the pressing surfaces conformed to that of the fruit and all parts coming in contact with the juice were of aluminum alloy.

**METHOD NO. 3.**—The juice was pressed from the whole peeled fruit by means of a conical worm in a conical fluted housing having a strainer slot

along the bottom and a valve at the end through which the pulp passed. This press was made of iron heavily tinned, except on the edges of the worm and the flutings, and these were kept bright.

**METHOD NO. 4.**—The pulp of the fruit was reamed out on a high-speed revolving cone (9) of a stainless alloy and collected in a porcelain bowl.

#### AFTER-TREATMENT

In handling the juice after extraction and prior to storage the procedure was as follows: The juice was run directly from the extractor through a tinned wire strainer with 18 meshes to the inch and then into a glass receptacle from which the juice was transferred to a glass vacuum flask and vacuumized (41). A vacuum was kept on the flask, and as 200–300 cc. of juice were collected in the upper container it was transferred to the flask by opening the glass stopcock. In this way the juice was under vacuum within 2 or 3 minutes, at the longest, after it had been extracted. Juice extracted by method no. 4 was subject to excessive foaming during the vacuumizing process, which suggested the possibility that a great deal of air had been dissolved by the beating effect of this method. While the exact importance of the use of the vacuum is not established, it has the advantage in experimental work of giving samples of juice that contain approximately the same amounts of dissolved air, thus eliminating marked variations that might be due to unequal aeration. The vacuum in the flask was relieved with air admitted to the top of the flask (not bubbled through the juice).

The suitability of glass containers for frozen-pack work has been discussed by WOODROOF (45). After extraction and treatment the juice was placed in 8 or 10-oz. jelly glasses and capped under vacuum with lacquered caps. A vacuum of 27–29 inches was drawn on the container for an instant at the time of capping.<sup>1</sup> These containers were selected as the best type for experimental work because they eliminated the possible effects of paraffin, paper, air, and absorbed odors. The 8-oz. container was usually used and filled to 0.25 inch of the top.

The juice was frozen by immersing the glasses up to the lids in rapidly circulating brine, and then placing upon the lids a metal tank through which also brine was circulating. The brine temperature was maintained at  $-20^{\circ}$  to  $-25^{\circ}$  F., and the contents of the glasses were completely frozen in about 25 minutes. After freezing, the outsides of the glasses were rinsed in fresh water to remove the brine and they were stored at  $0^{\circ}$  F.

Some of the juice was put into similar containers, and without previous freezing was stored at  $32^{\circ}$  F. The samples of frozen juice were thawed by immersing the containers in running tap-water, and the caps were removed when thawing was completed. The fruit was washed prior to ex-

<sup>1</sup> Equipment for capping the containers lent by courtesy of the manufacturers.

tracting the juice and all containers and extractors were kept thoroughly clean but were not sterilized.

#### PHYSICAL AND CHEMICAL DETERMINATION

When juice was first extracted, detailed records were made of effective acidity (pH), total soluble acids, total sugars, and other physico-chemical characters. Such determinations were made again whenever samples were removed from storage for testing. The quinhydrone electrode was used in making the pH determinations (35). Total soluble acids were determined by titration with sodium hydroxide, using phenolphthalein as an indicator, corrections being made for temperature differences. The results are expressed as percentage by weight of anhydrous citric acid of the expressed juice.

Specific gravity was determined by means of an hydrometer reading to 0.001, corrections being made for temperature differences. The results are expressed at  $\frac{60^\circ}{60^\circ}$  F.,<sup>2</sup> and have also been converted to the corresponding approximate values for degrees Brix for purposes of comparison with results appearing in the literature (8, 11, 37, 38).

Naringin was prepared from grapefruit according to the method of ZOLLER (46); hesperidin was prepared from the sweet orange according to the method of WANDER (42). The words "glucoside" and "glucosidal" as used in this paper refer to the fractions extracted by these methods. In the case of the characteristic glucoside of Satsuma and of lime, the procedure of WANDER was followed and the purified product obtained was assumed to be the glucoside.

#### Presentation of data

The principal changes observed in prepared citrus juices on aging as related to methods of preparation are those in taste, color, and movement of larger particles in the more or less translucent or transparent suspension medium. While working with Kawano Wase Satsuma oranges on October 19, 1931, a definite correlation was established between certain methods of preparation and the development of a bitter taste and a change in color (table I). In addition to these problems, changes in the direction and rate of movement of suspended particles challenged investigation (39, 6).

The effective acidity (pH) and the total soluble acids and solids of the mixture gave no indications as to the causes of the observed changes as shown by the data in table II. Although there were preceptible differences with regard to these characters, there seems to be no definite correlation between the observed changes in the juice and the differences mentioned.

<sup>2</sup> Indicates density at 60° F., referred to water at the same temperature.

TABLE I

CORRELATION BETWEEN METHOD OF PREPARATION AND DEVELOPMENT OF BITTER TASTE AND CHANGE IN COLOR; KAWANO WASE SATSUMA ORANGES, OCTOBER 19, 1931

METHOD OF PREPARATION*	CHANGE IN		REMARKS
	TASTE	COLOR	
No. 1a (check)†	Pleasantly tart; no bitter taste	Deep chrome (orange); no change	Retained good quality in refrigerator for several days
No. 2..	Pleasantly tart with added flavor of citrus oil; no bitter taste	Color changed from deep chrome (orange) to sulphur (light yellow) within 1 hour	Did not develop bitter taste on standing at room temperature for 24 hours
No. 3	Developed bitter taste in 2 hours	Deep chrome (orange); no change	Taste became exceedingly bitter after 4 hours
No. 4.	Developed bitter taste in 2 hours	Deep chrome (orange); no change	Taste became exceedingly bitter after 4 hours

\* See text for descriptions of methods of preparation.

† On all later experiments, method no. 1 was used as the check treatment.

It was therefore necessary to make an attempt to localize the causes, either in the external environment or in the complex tissues of the fruit, or in a combination of these two factors. After localization of the causes the next logical step was to determine the factors involved in bringing about the changes.

The results are presented in order as follows: (a) changes in taste; (b) changes in color; and (c) changes in direction and rate of movement of suspended particles.

#### CHANGES IN TASTE

Undesirable tastes developed in prepared citrus juices on aging are described as *stale*, *bitter*, *limy*, *musty*, and "turpentine" in the literature as reported up to 1925 (28, 13). These terms may not be strictly applicable to the product put up under the new frozen-pack method. In the case of juices put up in glass containers by the new method, one of the chief difficulties encountered up to the present is the development of a bitter taste (9). As shown in tables I and IX, this bitter taste develops relatively soon after the extraction of the juice, if the method of preparation is a predisposing factor. However, such other factors as the temperature of storage and the maturity of the fruit must also be considered in this connection. While other undesirable flavors may develop in the frozen or cold-stored juice, this preliminary report deals only with the bitter taste.

**TASTING TECHNIQUE.**—As a rule the results presented are based upon the tasting of the juices by two or more individuals. An attempt was

TABLE II  
CHARACTERISTICS OF CITRUS JUICES AND THE EFFECT OF AFTER-TREATMENT

TYPE OF CITRUS FRUIT	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	STORAGE PERIOD	REACTION	TOTAL ACIDS	SPECIFIC GRAVITY	DEGREES BRIX
Satsuma (Owari)*	11/19/31	1	Original juice	days	pH	per cent.		
"	"	1	Frozen	0	3.50	1.020	1.037	9.3
"	"	2	Original juice	6	3.51	0.996	1.036	9.0
"	"	2	Stored at 32° F.	0	3.52	1.000	1.037	9.3
"	"	2	Frozen	6	3.63	0.980	1.038	9.6
"	"	2	Original juice	6	3.46	0.980	1.037	9.3
"	"	3	Original juice	0	3.58	1.020	1.036	9.0
"	"	3	Stored at 32° F.	6	3.59	0.854	1.037	9.3
"	"	3	Frozen	6	3.59	0.860	1.037	9.3
"	"	4	Original juice	0	3.55	0.972	1.038	9.6
"	"	4	Stored at 32° F.	6	3.53	0.942	1.038	9.6
"	"	4	Frozen	6	3.54	0.935	1.037	9.3
Orange (Pineapple)*	11/23/31	1	Original juice	0	3.46	1.260	1.047	11.7
"	"	2	Original juice	0	3.47	1.250	1.047	11.7
"	"	2	Stored at 32° F.	2	3.41	1.490	1.048	12.0
"	"	2	Frozen	2	3.45	1.380	1.047	11.7
"	"	3	Original juice	0	3.47	1.390	1.047	11.7
"	"	3	Stored at 32° F.	2	3.48	1.360	1.048	12.0
"	"	3	Frozen	2	3.38	1.330	1.048	12.0
"	"	4	Original juice	0	3.45	1.390	1.047	11.7
"	"	4	Stored at 32° F.	2	3.40	1.430	1.048	12.0
"	"	4	Frozen	2	3.41	1.360	1.045	11.2
Tangerine (Dancy)	11/23/31	2	Original juice	0	2.95	2.150	1.048	12.0
"	"	2	Stored at 32° F.	4	2.95		1.047	11.7
"	"	2	Frozen	4	3.02	2.060	1.045	11.2
"	"	3	Original juice	0	2.96	2.060	1.048	12.0
"	"	3	Stored at 32° F.	4	3.07	2.010	1.047	11.7
"	"	3	Frozen	4	3.09	2.010	1.045	11.2
"	"	4	Original juice	0	3.03	2.120	1.048	12.0
"	"	4	Stored at 32° F.	4	3.03	2.000	1.048	12.0
"	"	4	Frozen	4	3.04	2.030	1.045	11.2
Lime (Key)*	7/23/31	2	Frozen	120	2.26	7.080	1.037	9.3
"	"	3	Frozen	120	2.31	6.650	1.037	9.3
"	"	4	Frozen	120	2.32	6.870	1.038	9.6

\* Similar data were compiled for Kawano Wase Satsuma; Valencia, Parson Brown, Washington Navel, and Hamlin Sweet Orange; and Tahiti lime.

† Two samples of shorter storage period gave same results.

made to use constant quantities of materials for tasting purposes in any particular experiment. The degrees of taste differences are indicated on a relative basis only. When any particular taste was present in a pronounced degree, it is indicated by a (+) sign. Decreasing degrees of any particular taste are indicated by (+-), (+--), etc.; increasing degrees are indicated by (++) , (+++), etc.

**BITTER TASTE.**—A clue to the localized tissues responsible for the bitter taste which develops in citrus juice prepared by certain methods was definitely shown in preliminary experiments (39, 6). The difference in taste was found to be due to the method of preparation, and this indicated that the cause of the trouble could possibly be traced to certain of the complex tissues of the fruit. While still in the whole fruit, these various tissues are effectively separated from one another. As has been indicated, however, the juice extraction process tears down these barriers more or less completely and breaks up the tissues, depending on the method used, and the resulting juice may be a mixture of material from most of the tissues. Once the juice is prepared in a manner predisposed to the development of the bitter taste, other factors may enter which may delay or modify the degree of its development under any particular conditions of after-treatment.

Although it was suspected that the causal agent might be of glucosidal nature, the plan of procedure included a comprehensive study of other possible causes. The following lines of investigation were followed in an attempt to determine the source of the undesirable tastes: (a) localization of the cause in the tissues; (b) determination of the nature of the mechanism involved (whether enzymatic, non-enzymatic, solution, etc.); (c) determination of effect of variety, maturity, and quality of fruit; and (d) determination of effect of after-treatment of the juice (freezing, storage, etc.).

**LOCALIZATION OF CAUSAL AGENT OR AGENTS.**—It was necessary to consider as many of the tissues of the fruit as possible so that no important source of the trouble would escape investigation. The tissues which were obviously of no importance were quickly eliminated, but a more detailed study of the remaining tissues was made with the object of localizing the cause or causes in a definite region or regions in the fruit.

To eliminate from the study the tissues which did not seem to contain the causal agent of the bitter taste, infusions were made by adding macerated tissues to the juice and testing for the effect on bitter taste development. The check treatments consisted of similar dilutions using tissue infusions made with water. Since the degree of dilution necessary to secure any marked results was unknown, three degrees of dilution were included in the first experiment, 2, 4, and 8 parts of macerated tissue to 100 parts of juice pressed from juice sacs only.

TABLE III

LOCALIZATION IN TISSUES OF SATSUMA ORANGE FRUIT (KAWANO WASE) OF THE CAUSE OF THE BITTER TASTE, OCTOBER 28, 1931 (DILUTIONS MADE WITH JUICE PRESSED FROM JUICE SACS ONLY)

INFUSION		PARTS INFUSION PER 100 PARTS LIQUID	BITTER TASTE DEVELOPMENTS*	
TISSUE	EXTRACTIVE USED		IMMEDIATE	LATER
Outer peel	Water	2	-	-
	"	4	-	-
	"	8	+ -	+ -
	Juice	2	-	-
" "	"	4	+ -	+ -
	"	8	+ -	+
Inner peel and veins	Water	2	+ -	+ -
	"	4	+ -	+ -
	"	8	+	+
	Juice	2	+ -	+ -
" " " "	"	4	+ -	+ -
	"	8	+	+
Locular walls	Water	2	+ - -	+ -
	"	4	+ -	+
	"	8	+	++
	Juice	2	+ -	+ -
" "	"	4	+ -	+ -
	"	8	+	+
Central axis	Water	2	-	-
	"	4	-	-
	"	8	-	-
	Juice	2	-	-
" "	"	4	-	-
	"	8	-	-
Seed coat	Water	2	-	-
	"	4	-	-
	"	8	-	-
	Juice	2	-	-
" "	"	4	-	-
	"	8	-	-
Cotyledons and germs	Water	2	-	-
	"	4	-	-
	"	8	-	-
	Juice	2	-	-
" " "	"	4	-	-
	"	8	-	-

\* Relative taste differences are indicated as follows: (-) indicates absence of the bitter taste; (+-), (+), (++), etc., indicate relatively greater degrees of the bitter taste.

The data in table III show that the tissues most probably responsible for the development of the bitter taste are the inner peel and veins, the outer peel, and the locular walls (3). The respective degrees of bitterness developed under the experimental conditions (dilution 8 parts of macerated tissue to 100 parts of juice) were for the freshly prepared infusion of outer peel (+-), and for the same infusion after standing (+); for the inner peel and locular walls (+) when freshly prepared infusion

was tested, and (+) for the same infusion after standing. The tastes given by the seed coat and cotyledons plus germs were of a nature different from the one being investigated; the central axis and juice-sac tissues were practically tasteless excepting in certain varieties of such citrus types as the grapefruit (tables VI, VII, and VIII). In this fruit the vascular tissues connecting with the juice sacs are much coarser than in some other citrus types, and the juice expressed from the separated locules thus carries more of the material commonly associated with the inner peel and locular wall. The juice sacs themselves are relatively coarse in structure and may contain the bitter principle. Similar results for the grapefruit have been reported by FELLERS (16). After the elimination of other tissues, the work was concentrated on the tissues of the inner peel, locular walls, and outer peel.

In further experiments, infusions made with outer peel from which the adhering portions of the inner peel had been more carefully removed failed to give a detectable bitter taste, although this taste may have been present but masked by the oil taste. As a result of these experiments, the outer peel was eliminated from the study as not being important in producing the bitter taste. Up to this point all the fruit used was below full maturity. In later experiments with riper fruit it appeared that the locular walls were probably less important as a source of the bitter taste than were the inner peel and the vein tissues.

The next step was to determine the factors involved in bringing about undesirable taste developments in prepared citrus juices from the standpoint of the presence or absence of inner peel and veins or locular-wall tissue.

The tests for the enzymatic factors proved negative. The work of REED (34) and McDERMOTT (41) on the inactivation of oxidases and peroxidases in citrus juices was verified as shown in table IV. The experiment included a study of: (a) the distribution of oxidase and peroxidase in the locular walls, inner peel and veins, and outer peel (29); (b) the effectiveness of water and juice in extracting possible enzymes; (c) the effect of Berkefeld filtering and centrifuging on tissue extracts made with water and juice; and (d) the effect on oxidase and peroxidase activity of mixing water extract of inner peel plus veins (giving positive reaction for oxidase and peroxidase) with the original juice, Berkefeld-filtered juice, and centrifuged juice.

In these experiments, all water extracts, untreated, Berkefeld-filtered, and centrifuged, gave positive evidence of oxidase and peroxidase activity; however, the last two gave color reactions to a less degree. Juice extracts as a general rule gave either negative or contradictory results; untreated juice and centrifuged juice extracts gave negative tests for these enzymes,

TABLE IV  
REGIONAL DISTRIBUTION OF OXIDASE AND PEROXIDASE IN THE TISSUES OF THE SATSUMA ORANGE FRUIT (KAWANO WASE);  
NOVEMBER, 2, 1931

TISSUE EXTRACTED	INFUSION*		OXIDASE (GUALACUM TEST)		PEROXIDASE (GUALACUM TEST)		REMARKS
	EXTRACT- ANT USED	AFTER-TREATMENT	NOT BOILED	CHECK (BOILED)	NOT BOILED	CHECK (BOILED)	
Locular walls	Water	Untreated	Blue	Negative	Intense blue	Negative	
"	"	Berkefeld- filtered	Light blue	"	Medium blue	"	
"	"	Centrifuged	Faint blue	"	Medium blue	"	
"	Juice	Untreated	Negative	"	Negative	"	
"	"	Berkefeld- filtered	Faint blue	"	Light blue	"	
"	"	"	"	"	"	"	
Inner peel and veins (a)	Water	Centrifuged	Negative	"	Negative	"	Same filter used as in water sample
"	"	"	"	"	"	"	
"	Water	Untreated	Blue	"	Intense blue	"	
"	"	Berkefeld- filtered	Faint blue	"	Very light blue	"	
"	"	"	"	"	"	"	
"	Juice	Centrifuged	Faint blue	"	Medium blue	"	
"	"	Untreated	Negative	"	Negative	"	
"	"	Berkefeld- filtered	"	"	"	"	
"	"	Centrifuged	"	"	"	"	
Outer peel	Water	Untreated	Faint blue	"	Intense blue	"	Not kept in cold storage
"	"	Centrifuged	Light blue	"	Intense blue	"	Not kept in cold storage
"	Juice	Untreated	Negative	"	Medium blue	"	
"	"	Centrifuged	"	"	"	"	
Other mixtures tested							
Original juice (b)		Berkefeld- filtered	"	"	Negative	"	
Original juice (c)		Centrifuged	"	"	"	"	
" (d)		Untreated	"	"	"	"	
Juice poured off parings of inner peel and veins			"	"	"	"	
Juice poured off locular walls			"	"	"	"	
Mixture of (a) and (b) †			"	"	"	"	
" (a) and (c) †			"	"	"	"	
" (a) and (d) †			"	"	"	"	

\* Infusions made in the proportions of 1 gm. of fresh tissue to 1 cc. extractant.

† Dilution: 1 part infusion to 8 parts of juice.

except in the case of outer peel extract; and Berkefeld-filtered juice extract gave contradictory results. Water extracts, whether Berkefeld-filtered, centrifuged, or untreated, when mixed with original Satsuma juice gave no indication of the presence of enzymes, indicating that the degree of acidity (pH 3.5 in this case) inactivates the enzymes present in the tissue extracts.

The presence of glucosidases was investigated from the viewpoint of possible change in optical activity of clarified juice from juice sacs only. When tissue infusions possibly containing glucoside-splitting enzymes were added to this clarified juice (19), any changes in optical activity observed were well within the probable error of the experiment.

The possible relation between other enzymes and the development of bitter taste was tested by adding boiled tissue to unboiled juice, *vice versa*, and also by boiling both before mixing. The tests gave negative results as shown in table V. There was no decrease but rather an increase of the bitter taste due to boiling.

TABLE V

TESTS TO DETERMINE THE NATURE (ENZYMATIC OR NON-ENZYMATIC) OF THE BITTER TASTE DEVELOPMENT IN CITRUS JUICES

INFUSION		BITTER TASTE* DEVELOPED	
EXTRACTANT USED	INNER PEEL AND VEINS ADDED	SATSUMA ORANGE (KAWANO WASE) Nov. 9, 1931	SWEET ORANGE (PARSON BROWN) Nov. 10, 1931
	gm.		
Juicet	None	-	-
"	0.01	-	+ -
"	0.10	-	+ -
"	1.00	+	+
"	5.00	++	++
"	1.00 boiled†	+	+
Boiled juicet ‡	None	- (+ boiled taste)	- (++ boiled taste)
"	1.00	++ (+ boiled taste)	+ - (++ boiled taste)
"	1.00 boiled‡	+++ (+ boiled taste)	++ (++ boiled taste)
Water	None	-	-
"	0.01	+ -	+ - -
"	0.10	+	+
"	1.00	++	+
"	5.00	+++	++
"	1.00 boiled‡	++	+++

\* Relative taste differences are indicated as follows: (-) indicates absence of the bitter taste; (+-), (+), (++) , etc., indicate increasing degrees of bitter taste. Data taken within 15 minutes after mixing and re-checked after 24 hours.

† Juice pressed from juice sacs only.

‡ Heated to boiling point.

The evidence apparently points to the possibility of a non-enzymatic cause for bitterness. Experiments to test this hypothesis were conducted along four lines: (a) testing the possible identity of the causal agent for bitter taste with glucosides; (b) determining the effect on taste of varying amounts of glucoside; (c) determining the effect of the extraction pressure and the temperature factors on the degree of bitter taste developed in prepared citrus juices; and (d) determining the solubility of the substance causing the bitter taste.

Aliquots of the tissue extracts were centrifuged and Berkefeld-filtered; similarly, aliquots of the juice used for dilution in the case of locular walls and inner-peel and vein infusions were centrifuged and Berkefeld-filtered. The centrifuging and Berkefeld-filtering processes did not seem to remove the causes of bitterness, since samples consisting of Berkefeld-filtered infusions diluted with similarly treated juice expressed from juice sacs only still gave mixtures which developed a bitter taste. As a result of these experiments it appeared that the cause of the bitter taste was a soluble substance.

When the experiments were undertaken, it was suspected that the causal agent for the bitter taste was of glucosidal origin; however, it was necessary to eliminate other possibilities in order to obtain reasonable assurance that the original hypothesis might prove tenable.

The data presented in tables V and VI indicate that the degree of bitterness developed when inner-peel and vein tissue or naringin is added to Satsuma juice pressed from juice sacs only is in a marked degree proportional, within the limits of the experiment, to the amount of inner-peel and vein tissue or glucoside added to juice expressed from juice sacs only. In the case of grapefruit, the bitter taste is present even in juice expressed from juice sacs only, but the degree of the characteristic bitterness may be increased by adding naringin, as shown in table VI.

A more comprehensive experiment which verifies these conclusions was carried out with juice of Satsuma orange, sweet orange, grapefruit, lime, and tangerine, as presented in table VII. In the experiment the juice from juice sacs only was treated in two ways: (a) by addition of the glucoside extracted from the inner peel and veins; (b) by addition of the inner peel and veins from which the glucosides had been extracted. The data show that the extracted tissues gave negative results except in the case of grapefruit juice, which result is in harmony with previous experiments. The addition of the extracted glucoside gave positive results, the degree of bitterness increasing with the amount of glucoside added.

The data presented in tables V, VI, and VII indicate that the major cause of the bitter taste developed in prepared citrus juices on aging apparently is of glucosidal origin. Since the chemical structure of the



TABLE VII

EFFECT OF CHARACTERISTIC GLUCOSIDE AND GLUCOSIDE-FREE TISSUE ON THE DEVELOPMENT OF THE BITTER TASTE IN PREPARED CITRUS JUICES.\*  
JUICE PRESSED FROM JUICE SACS ONLY

VARIETY	SOLVENT	TASTE OF UNTREATED SOLVENT	TASTES DEVELOPED WHEN VARYING AMOUNTS OF GLUCOSIDE WERE ADDED			TASTES DEVELOPED WHEN VARYING AMOUNTS OF GLUCOSIDE-FREE TISSUE WERE ADDED		
			1/100 SATURATED	1/10 SATURATED	SATURATED	WEIGHT	TASTE	WEIGHT
Satsuma (Owari) Dec. 4, 1931	Juice	-	+	+	+	gm. 0.25	-	gm. 0.25
	Water	-	+-	+	++	0.01	-	0.25
Orange (Parson Brown) Nov. 12, 1931	Juice	-	-	+-	+	0.10	-	1.00
	Water	-	-	+-	+	0.10	-	1.00
Orange (Hamlin) Dec. 4, 1931	Juice	-	+-	+-	+-	0.25	-	1.00
	Water	-	+-	+-	+	0.25	-	1.00
Grapefruit (Silver Cluster) Dec. 4, 1931	Juice	+	+	++	++++	0.25	+	1.00
	Water	-	+	++	++++	0.25	-	1.00
Lime (Key) Dec. 4, 1931	Juice	-	+	++	++++	0.01	-	0.25
	Water	-	-	++	++++	0.01	-	0.25
Tangerine† (Dancy) Dec. 4, 1931	Juice	-	+	+	++++	0.25	-	1.00
	Water	-	-	+	++++	0.25	-	1.00

\* Relative taste differences are indicated as follows: (-) indicates absence of the bitter taste; (+-), (+), (++) , etc., indicate relatively greater degrees of the bitter taste.

† Tissue before extracting (1 gm.) gave +.

‡ Naringin was used; characteristic tangerine taste present in all juices.

glucosides contained in various types of citrus is not the same, it would be expected that the bitter tastes associated with them would not be identical. This is actually the case; and so far as the work was carried, each glucoside or closely related group can be recognized by its characteristic taste qualities.

The data presented in tables V and VI show also that the temperature factor may be of importance with reference to the rate of bitter taste development and the final value attained. The figures in table V show that the degree of bitter taste developed is markedly greater in cases where either the juice, the tissue, or both are heated before or after mixing. The figures presented in table VI, although not so conclusive as those presented in table V, show that apparently more naringin goes into solution as the temperature is raised, as indicated by increased bitterness of taste after boiling. This is in harmony with the work of ZOLLER (46), who has shown that naringin is more water-soluble at higher temperatures.

The data presented in table VIII indicate that the degree of bitterness developed when juice is expressed from inner-peel and vein tissue with attached juice vesicles by means of porcelain plates is in a marked degree correlated with the pressure exerted. The first 100 cc. develop the least bitter taste and usually the degree of bitterness rises markedly in the case of the third 100 cc. (14). It should be pointed out that when juices are expressed from juice sacs only, except in the case of the grapefruit, a bitter taste does not normally develop, even in 8 or 9 days in cool storage. This would indicate that the data in table VIII possibly should be interpreted on the basis that the bitter taste is due to dissolved glucosidal material pressed primarily from the cells of the inner peel and veins and locular wall tissues.

TYPE, VARIETY, QUALITY, AND MATURITY FACTORS.—The data presented in tables I to VIII, inclusive, are in harmony with the commonly accepted ideas regarding the wide differences in kind of juice secured from various types of citrus fruits, such as Satsuma orange, sweet orange, tangerine, grapefruit, and lime. The chief variations, such as effective acidity (pH), total soluble acids and solids, as well as the more subtle taste qualities associated with each type, are due to the kind of citrus fruit used. Within a citrus type the effect of variety on the quality of the juice is of great importance.

In the case of the Satsuma orange, two varieties have been studied. With juice prepared on October 19 from Kawano Wase Satsuma oranges (table I) a marked bitter taste developed within two hours. With juice of Owari satsumas the results were somewhat different. Juices were prepared on November 19 from fruits of average maturity from Marianna (table IX), and fruits of advanced maturity from the station orchard at Gainesville on

TABLE VIII

EFFECT OF VARIOUS DEGREES OF PRESSURE AND STAGE OF MATURITY ON DEVELOPMENT OF BITTER TASTE IN PREPARED CITRUS JUICES

TYPE OF CITRUS FRUIT	DATE	STATE OF MATURITY	NATURE OF TISSUE	PRESS-INGS†	TASTE DEVELOPMENTS*				
					INITIAL	AFTER 4 HRS.	AFTER 8 HRS.	AFTER 12 HRS.	AFTER 20 HRS.‡
Satsuma (Owari)	Dec. 3, 1931	Relatively mature	Inner peel and locular walls	1st 2nd 3rd	- +-- +--	- +-- +--	+-- +-- +--	+-- +-- +--	+ + +
Orange (Hamlin)	"	"	Locular walls	1st 2nd 3rd	- - -	- - -	- - -	- - -	- - -
"	"	"	Inner peel and veins	1st 2nd 3rd	- - -	- - -	- - -	- - -	+-- +-- +--
"	"	"	Locular walls and inner peel and veins	1st 2nd 3rd	- - -	- - -	- - -	- - -	+-- +-- +--
Tangerine (Dancy)	Dec. 3, 1931	Relatively immature	Locular walls	1st 2nd 3rd	+ + ++	+ + ++	+ + ++	+ + ++	+++ +++ ++++
"	"	"	Inner peel and veins	1st 2nd 3rd	+ + +	+ + +	+ + +	+ + +	+++ +++ +++
Grapefruit (Walters)	Nov. 18, 1931	Relatively immature (from Station grounds)	Juice sacs only	1st 2nd 3rd	+ +++ ++++	+ +++ ++++	+ +++ ++++	+ +++ ++++	+ +++ ++++

\* Relative taste differences are indicated as follows: (-) indicates absence of a bitter taste; (+-), (+), (++) , etc., indicate relatively greater degrees of the bitter taste.

† 1st indicates first 100 cc. of one pressing; 2nd indicates the second 100 cc. of the same pressing; 3rd indicates juice sample obtained by repressing. Pressings made between porcelain plates.

‡ Juice stored at 40° to 50° F.

December 3 (table VIII). In the sample of average maturity prepared by methods 3 and 4 a bitter taste was developed to the degree of (+) at the end of two and three hours, respectively (table IX); the sample of advanced maturity gave a bitter taste only to the degree (+ - -) even when the juice was pressed from inner-peel and vein parings as indicated in table VIII.

As shown in table IX, the Washington Navel is more likely to develop the bitter taste than Parson Brown, Pineapple, or Hamlin. In grapefruit the glucoside taste is expected and there is even a popular prejudice against such a variety as Triumph which does not have the characteristic glucoside bitterness of this type of citrus. The study of the effect of quality of fruit within a variety in this particular has not been undertaken experimentally.

In most cases the bitter taste developed in sweet orange juice was found to be relatively little and was found to decline with advancing maturity. In table IX it will be noted that on November 13 and 14 two samples of juice were prepared from Parson Brown oranges, the first with fruit from Lady Lake, Florida, and the second with much less mature fruit from Lake Alfred. The first sample developed no noticeable bitter taste but the second sample developed a degree of bitterness with methods 3 and 4 of (+ -) after two hours of standing, indicating the effect of maturity in decreasing bitterness. Pineapple and Hamlin orange juice prepared by methods 3 and 4 gave results very similar to these. Juice prepared from the Washington Navel variety, however, on November 14 and 19 developed a degree of bitterness with methods 3 and 4 of (+ +) and (+), respectively, after two hours' standing on the first date and a degree of bitterness amounting to (+) with both methods after standing for five hours for the second date (table IX).

The tangerine juices prepared on November 23 gave results closely paralleling those for satsumas, developing a very bitter taste for methods nos. 3 and 4 after standing for several hours (tables I, VIII, and IX). Tahiti and lime juices prepared on November 18 by methods 3 and 4 developed a disagreeably bitter taste (table IX).

The results discussed are of course a mere beginning toward the complete listing of citrus varieties suitable for juice production on the basis of glucoside content and other constituents and their relative proportions remaining after various stages of maturity. The subject is of major importance and will require comprehensive experiments.

The preliminary results are in harmony with those often quoted in the literature to the effect that a bitter taste does not always develop or that properly matured fruit does not give a poor quality juice (28, 9, 12). The experiments here reported support these empirical statements and will make it possible after the subject is thoroughly investigated to select citrus fruits intelligently so as to minimize the bitter taste in the product. In determining the exact range in the maturity of citrus fruits when materials should

# BITTER TASTE DÉVELOPMENTS IN PREPARED CITRUS JUICES IN RELATION TO METHODS OF PREPARATION, FREEZING, AND STORAGE\*

TYPE OF CITRUS FRUIT	DATE PRE- PARED (1931)	METHOD OF PREPARA- TION	AFTER-TREAT- MENT	STORAGE PERIOD	BITTER TASTE DEVELOPMENTS AT DIFFERENT SAMPLINGS. FIGS. IN ( ) REFER TO HOURS AFTER EXPERIMENT WAS SET UP							
					1ST	2ND	3RD	4TH	5TH	6TH		
Satsuma (Owari)	Nov. 19	1	Original juice	days								
	"	1	Frozen	0	-	(4)-		(3)-			(16)-	
	"	2	Original juice	6	-	(4)-		(3)-	(5)-			
	"	2	Stored at 32° F.	0	-	(4)-	(2)-	(3)-	(4)-			
	"	2	Frozen	6	-	(4)-	(1½)-	(3)-	(4)-			
	"	3	Original juice	0	-	(4)+	(2)+	(3)+	(5)+			
	"	3	Stored at 32° F.	6	+-	(4)+	(1½)+	(3)+	(5)+			
	"	3	Frozen	"	+-	(4)+		(3)+	(5)+			
	"	4	Original juice	0	-	(4)+	(1½)+	(3)+	(5)+			
	"	4	Stored at 32° F.	6	+-	(4)+	(1½)+	(3)+	(5)+			
	"	4	Frozen	6	-	(2)-		(3)-	(5)+			
	Satsuma (Wase)	Nov. 13	2	Original juice	0	-	(2)-		(3)-			
"		2	Stored at 32° F.	2	-	(3)-		(3)-				
"		3	Original juice	0	+	(2)+		(3)+				
"		3	Stored at 32° F.	2	++	(3)+		(3)+				
"		4	Original juice	0	++	(2)+		(3)+				
"		4	Stored at 32° F.	2	+-	(3)+		(3)+				
Orange (Parson Brown) Lady Lake, Fla.		Nov. 13	2	Original juice	0	-	(2)-		(3)-			
		"	2	Stored at 32° F.	2	-	(2)-		(3)-			
	"	3	Original juice	0	-	(2)-		(3)-				
	"	3	Stored at 32° F.	2	-	(2)-		(3)-				
	"	4	Original juice	0	-	(2)-		(3)-				
	"	4	Stored at 32° F.	2	-	(2)-		(3)-				
	Orange (Parson Brown) Lake Alfred, Fla.	Nov. 14	2	Original juice	0	-	(2)-		(3)-			
		"	2	Stored at 32° F.	2	+-	(2)+		(3)+			
"		3	Original juice	0	+-	(2)+		(3)+				
"		3	Stored at 32° F.	2	+-	(2)+		(3)+				
"		4	Original juice	0	+-	(2)+		(3)+				
"		4	Stored at 32° F.	2	-	(2)+		(3)+				

TABLE IX—(Continued)

TYPE OF CITRUS FRUIT	DATE PRE- PARED (1931)	METHOD OF PREPARA- TION	AFTER-TREAT- MENT	STORAGE PERIOD	BITTER TASTE DEVELOPMENTS AT DIFFERENT SAMPLINGS. FIGS. IN ( ) REFER TO HOURS AFTER EXPERIMENT WAS SET UP					
					1ST	2ND	3RD	4TH	5TH	6TH
Orange (Washington Navel)	Nov. 19	2	Original juice	0	—		(1)–	(5)–	(6)–	
“	“	2	Stored at 32° F.	4	—			(5)–		
“	“	2	Frozen	6	—		(1) + – –	(5) + –		
“	“	3	Original juice	0	—	(4) + –		(5) +	(6) + +	
“	“	3	Stored at 32° F.	4	+		(2) + +			
“	“	3	Frozen	6	+	(4) ÷	(3) + +	(5) + + +		
“	“	4	Original juice	0	+	(4) + –		(5) +	(6) + +	
“	“	4	Stored at 32° F.	4	+		(2) + + +			
“	“	4	Frozen	6	+	(4) +	(2) + +	(5) + + +		
Tangerine (Dancy)	Nov. 23	1	Original juice	0	—	(4) –				
“	“	2	“	0	—	(4) –	(5) –	(7) –		
“	“	2	Stored at 32° F.	4	—	(3) –	(1) –	(3) –		
“	“	2	Frozen	4	—	(4) –	(1) + – –	(3) + –		
“	“	3	Original juice	0	+	(4) +	(6) + + + +			
“	“	3	Stored at 32° F.	4	+	(3) +	(1) +	(3) +		
“	“	3	Frozen	4	+	(4) + +	(1) + +	(3) + + +		
“	“	4	Original juice	0	+	(4) + +	(1) + +	(7) + + +		
“	“	4	Stored at 32° F.	4	+	(3) + +	(1) +	(3) +		
“	“	4	Frozen	4	+	(4) + +	(1) +	(3) + +		
Lime (Tahiti)†	Nov. 18	2	Original juice	0	—					
“	“	3	“	0	+					
“	“	4	“	0	+					
“	“	2	“	0	—					
Lime (Key)†	“	3	“	0	+					
“	“	3	“	0	—					
“	“	4	“	0	+					

\* Relative taste differences are indicated as follows: (–) indicates absence of a bitter taste; (+–), (+), (++) , etc., indicate relatively greater degrees of the bitter taste.

† Juice diluted 1 part juice to 10 parts water before tasting.

be selected for juice extraction, two opposing tendencies must be considered. While the glucoside content decreases with age (46), as a general rule the processes of senescence become increasingly important and lead to other undesirable qualities than the bitter taste of the prepared product. The exact range within which the fruit should be selected will depend not only on the variety but also on the local environmental differences which would tend to shift the period in one direction or the other. The rule to be followed in each case would be to harvest the fruit when the glucoside content has decreased sufficiently to reduce to a minimum the tendency of the product to develop a bitter taste and before the processes of senescence have proceeded far enough to bring other undesirable qualities into the product.

EFFECT OF AFTER-TREATMENT OF JUICE ON DEVELOPMENT OF BITTER TASTE.—The vacuumized juice was poured into 8-oz. jelly glasses and again vacuumized during the sealing process. The product was then stored in two ways, at 32° F. (0° C.) without preliminary freezing and at 0° F. (−18° C.) with preliminary freezing. Samples were then removed at convenient intervals for investigation. The first treatment was employed to test the possibility of preserving the juices for short periods, up to 36 hours, in order to meet the demand of the producer who wishes to market unfrozen citrus juices. The second treatment was intended to meet the demand for a feasible method of storing the product over a period up to eight months or longer.

In the case of unfrozen juice stored at 32° F. (0° C.) for short periods of time, satisfactory results have been obtained with methods 1 and 2, so far as controlling the bitter taste is concerned (table IX). In some instances, however, other undesirable taste qualities have given some trouble; therefore method 2 was feasible with these exceptions. With methods 3 and 4, as a general rule the development of the bitter taste could not be effectively controlled even by immediate storage, except in cases where the glucoside was present in minimal quantity. As shown in table IX, the rate and degree of development of the bitter taste were not consistently affected by the treatment. In this connection it should be pointed out that some of the undesirable taste, especially when present in minor quantities, was not readily distinguished when the product was consumed in the cold condition. This indicates that the undesirable taste would likely be less pronounced in actual commercial practice if the product were produced and marketed under low-temperature conditions. It would be necessary to indicate to the consumer, however, that the product might deteriorate rapidly at ordinary room temperatures.

When citrus juices were frozen and stored at 0° F. (−18° C.), partially satisfactory results were obtained in all cases by the use of methods 1 and 2. Valencia orange and Tahiti lime juices put up by method 2 developed no

bitter taste even after four months of storage at 10° (−12° C.) in the frozen condition. Lime juice prepared according to method 4, however, after four months' storage developed a perceptibly bitter taste within an hour after defrosting. The results secured by storing juices in the frozen condition at 0° F. (−18° C.) for shorter periods in the case of various types of citrus, including Satsuma orange, sweet orange, tangerine orange, and lime, were similar to those heretofore reported for Valencia orange and Tahiti lime over a longer period.

**OTHER TASTE QUALITIES.**—Of the various other taste qualities, desirable and otherwise, only that imparted by the addition of citrus oil is taken up in this report. A minimum addition of citrus-peel oil to the freshly extracted juice is not objectionable and in fact is usually preferred. While the presence of a small amount of oil has not had any bad effect on juice frozen by certain methods, it might result in undesirable taste qualities under some other methods of freezing and storage. After four months' storage, Valencia orange and Tahiti lime juice extracted by method 2 and frozen and stored under vacuum had only the very slightest off-taste, although there was considerable oil content. As pointed out in the preceding discussion, a minimum amount of citrus oil may entirely mask the bitter taste developed in juice prepared by some methods of manufacture.

#### CHANGES IN COLOR

Second in importance to taste is the color of prepared citrus juices. The orange or yellow coloring matter of the flesh of various citrus types is localized in small bodies (plastids) found in the cells which compose the juice sacs of the fruit (26). The plastids vary in shape with the type of citrus.

The red, orange, and yellow pigments occurring in plants fall into several chemical classes: carotinoids, flavones (anthoxanthones), and anthocyanins (19, 31, 43, 44). The presence of the carotinoids has been demonstrated in certain citrus juices by MATLACK (27). The color changes in citrus juices were studied on two types of material, empty juice sacs, and the juice containing the extracted plastid pigments.

Color descriptions are based on the MAERZ and PAUL color dictionary (25). Two types of material were described as to color. In one case the pigments were in solution and in the other the colored particles were suspended in the juice mixture. An 8-oz. tumbler was filled to a depth of 0.75 inch, and the mask was placed over the top of the tumbler which was set on a 600-cc. beaker inverted over a white background. This allowed light to penetrate from all sides, making the optical conditions comparable with those of a liquid in a bottle. When the color was in solution it was not practicable to use this method, and then color comparisons were made by examination of the liquid in the tumbler from the side without a mask. In report-

**TABLE X**  
SOLUBILITY TESTS ON PIGMENTS OF EMPTY JUICE SACS OF VARIOUS CITRUS TYPES

SOL- VENT GROUP*	SATSUMA (OWARI)				SWEET ORANGE (HAMLIN)				TANGERINE (DANCY)			
	SOLU- BILITY IN 50 CC. SOL- VENT	ORIGINAL COLOR OF SACS (10 GM.)	COLOR OF SACS AFTER TREATMENT	COLOR OF FILTRATE	SOLU- BILITY IN 50 CC. SOLVENT	ORIGINAL COLOR OF SACS (10 GM.)	COLOR OF SACS AFTER TREATMENT	COLOR OF FILTRATE	SOLU- BILITY IN 50 CC. SOLVENT	ORIGINAL COLOR OF SACS (10 GM.)	COLOR OF SACS AFTER TREATMENT	COLOR OF FILTRATE
I	Ethyl alcohol 95 per cent.	9-9-L† (Deep orange)	9-5-K (Yellow)	9-2-K (Yellow)	+	9-4-L (L. orange yellow)	9-2-K (Yellow)	9-1-I (Yellow)	++	9-9-I (Orange)	9-6-L (Orange)	9-4-K (Orange yellow)
		9-9-L (Deep orange)	9-3-K (Yellow)	9-2-K (Yellow)	+	9-4-L (L. orange yellow)	9-1-I (Yellow)	9-1-L (Yellow)	++	9-9-I (Orange)	9-5-K (Orange yellow)	9-2-K (Yellow)
	Acetaldehyde	9-9-L (Deep orange)	9-4-L (L. orange yellow)	9-2-J (Yellow)	+-	9-4-L (L. orange yellow)	9-1-L (Yellow)	10-1-C (Pale yellow)	++	9-9-I (Orange)	9-5-K (Orange yellow)	9-2-K (Yellow)
		9-9-L (Deep orange)	9-5-K (Orange yellow)	9-2-J (Yellow)	+-	9-4-L (L. orange yellow)	9-2-K (Yellow)	10-1-C (Pale yellow)	+	9-9-I (Orange)	9-2-K (Orange yellow)	9-2-K (Yellow)
	Ethyl ether	9-9-L (Deep orange)	9-4-L (L. orange yellow)	9-2-J (Yellow)	+-	9-4-L (L. orange yellow)	9-2-K (Yellow)	10-1-C (Pale yellow)	++	9-9-I (Orange)	9-4-L (L. orange yellow)	9-2-K (Yellow)
II	Chloroform	9-9-L (Deep orange)	9-4-L (L. orange yellow)	9-2-J (Yellow)	+-	9-4-L (L. orange yellow)	9-2-K (Yellow)	9-1-I (Yellow)	+	9-9-I (Orange)	9-3-K (Yellow)	10-1-I (Yellow)
	Petrol ether	9-9-L (Deep orange)	9-4-L (L. orange yellow)	9-1-J (Yellow)	-	9-4-L (L. orange yellow)	9-2-K (Yellow)	Colorless	+-	9-9-I (Orange)	9-3-K (Yellow)	9-2-J (Yellow)
III	Benzene	9-9-L (Deep orange)	9-4-L (L. orange yellow)	9-3-K (Yellow)	+-	9-4-L (L. orange yellow)	9-2-K (Yellow)	10-1-C (Pale yellow)	+	9-9-I (Orange)	9-4-L (L. orange yellow)	9-2-J (Yellow)
	Xylene	9-9-L (Deep orange)	9-4-L (L. orange yellow)	9-3-K (Yellow)	+-	9-4-L (L. orange yellow)	9-2-K (Yellow)	10-1-B (Very dim yellow)	+	9-9-I (Orange)	9-4-L (L. orange yellow)	9-3-K (Yellow)
	Toluene	9-9-L (Deep orange)	9-4-L (L. orange yellow)	9-3-K (Yellow)	+-	9-4-L (L. orange yellow)	9-2-L (Yellow)	10-1-B (Very dim yellow)	+	9-9-I (Orange)	9-4-L (L. orange yellow)	9-2-J (Yellow)
	Carbon disulfide	9-9-L (Deep orange)	9-4-L (L. orange yellow)	9-6-I (Light orange)	+-	9-4-L (L. orange yellow)	9-4-K (L. orange yellow)	9-2-B (Very dim orange)	+-	9-9-I (Orange)	9-8-J (Orange)	9-5-E (Pale orange)
		9-9-L (Deep orange)	9-8-I (Orange)	9-6-I (Light orange)	+-	9-4-L (L. orange yellow)	9-4-K (L. orange yellow)	9-2-B (Very dim orange)	+-	9-9-I (Orange)	9-8-J (Orange)	9-5-E (Pale orange)

\* Group I dissolves all pigments equally.  
Group II dissolves carotin and traces of others.  
Group III acts immediately between first and second groups.  
† MAREZ and PAUL number.

ing the color reactions, the general range of color change is indicated in parentheses below the common names.

**NATURE OF JUICE-SAC PLASTID PIGMENTS.**—The solubility tests made gave results in harmony with the earlier work of MATLACK on the nature of the plastid pigments in citrus fruits (27). The data covering the solubility tests with the usual carotinoid solvents are presented in table X. The results of the solubility tests for anthocyanins are given in table XI.

TABLE XI

SOLUBILITY OF FRESH PLASTID PIGMENTS OF VARIOUS CITRUS TYPES IN HOT WATER

TYPE OF CITRUS FRUIT	SOLUBILITY OF ORIGINAL PIGMENT IN 100 GM. OF EMPTY JUICE SACS	SOLUBILITY OF LIGHTER COLORED PIGMENT RE- SULTING IN JUICE-SAC TISSUE TO WHICH PEEL OIL HAS BEEN ADDED
Satsuma (Owari)	Solubility in 50 cc. solvent —*	—
	Original color of sacs 9-9-L (Deep orange)	9-4-L (Light orange yellow)
	Color of sacs after treatment 9-9-L (Deep orange)	9-4-L (Light orange yellow)
	Color of filtrate Colorless	Colorless
Orange (Hamlin)	Solubility in 50 cc. solvent —	—
	Original color of sacs 9-4-L (L. orange yellow)	9-1-L (Yellow)
	Color of sacs after treatment 9-4-L (L. orange yellow)	9-1-L (Yellow)
	Color of filtrate Colorless	Colorless
Tangerine (Dancy)	Solubility in 50 cc. solvent —	—
	Original color of sacs 9-9-I (Orange)	9-5-L (Orange yellow)
	Color of sacs after treatment 9-9-I (Orange)	9-5-L (Orange yellow)
	Color of filtrate Colorless	Colorless
Lime (Key)	Solubility in 50 cc. solvent —	—
	Original color of sacs 17-2-G (Green)	17-1-J (Light yellow)
	Color of sacs after treatment 17-2-G (Green)	17-1-J (Yellow)
	Color of filtrate Colorless	(Colorless)

\* (—) indicates absence of any particular pigment.

The data reported in table X, using juice-sac material from Owari Satsuma orange, Hamlin sweet orange, and Dancy tangerine, with the usual carotinoid solvents, show that the pigments contained in this material were markedly soluble in ethyl alcohol, acetone, acetaldehyde, and chloroform,

the solvents which, according to TSWETT (40), dissolve all the carotinoids equally. The solubility with petrol ether is as a rule slightly less or nil. According to TSWETT, petrol ether mainly dissolves carotin. The third group of solvents used consisted of those acting in an intermediate manner between the foregoing (40), benzene, xylene, toluene, and carbon disulphide. These tests as a rule showed marked solubility except in the case of sweet orange. With sweet orange the juice was very light in color, owing to the comparative immaturity of the fruit; with riper fruit a more definite reaction would probably be obtained with these solvents.

The solubility tests with the usual technique used for anthocyanins (30) show that the juice-sac pigments of Owari Satsuma orange, Hamlin sweet orange, Dancy tangerine, and Key lime were insoluble in hot water, indicating that anthocyanins are apparently absent.

COLOR CHANGES DUE TO CITRUS OIL AND OTHER COMPOUNDS.—As indicated in table 1, the possible effect of citrus oil on the juices prepared by method no. 2 was to change the original Satsuma orange color of the juice to yellow. This opened up the field of color changes in prepared juices. Since no very marked color changes were observed with methods 1, 3, and 4, it seemed logical to look for the cause of the color change in the outer peel which introduced the chief variation in method 2. It was noticed also that the original Satsuma orange color was changed to yellow as a result of neutralization and pasteurization of the juice mixture. The results of a typical experiment covering the effect of citrus oil, neutralization, and pasteurization on color changes in Owari Satsuma orange, Hamlin sweet orange, Dancy tangerine, Key lime, and McCarty grapefruit are presented in table XII. It will be noted that the original color of prepared juices, orange or yellow\* as the case may be, can readily be changed from orange to a lighter shade of orange, or to yellow, or from yellow to a lighter shade of yellow, by the three methods indicated. In the case of neutralization the color change is reversible within certain limits.

Apparently the source of the citrus peel oil, as shown in table XIII, does not affect the results, since the change takes place in all citrus types studied whether sweet orange, Satsuma orange, tangerine, grapefruit, or lime oil is used. It should be noted also that hand-pressed oil gives the same results as distilled oil. In the sap expressed from sweet orange peel from which the oil had been extracted, as shown in table XII, no characteristic change of color was observed in Satsuma, tangerine, and sweet orange juices. This indicated that the causal agent was present in the citrus oil and not in the rest of the outer peel tissue.

The data in table XIII show also that the degree of observed color change was, within certain limits (1, 2, and 10 parts per 1000 parts of juice), in a marked degree proportional to the amount of citrus oil added. The

TABLE XII  
COLOR CHANGES IN CITRUS FRUIT JUICES PRODUCED BY CITRUS OIL, ALKALI, AND PASTEURIZATION

TYPE OF CITRUS FRUIT	ORIGINAL COLOR		AFTER ADDITION OF 2 CC. OF DISTILLED CITRUS PEEL OIL TO 25 CC. OF JUICE		AFTER NEUTRALIZATION WITH 0.1 N NAOH		AFTER PASTEURIZATION AT 180° F. FOR 30 MINUTES	
	MAERZ & PAUL NUMBER	COMMON NAME	MAERZ & PAUL NUMBER	COMMON NAME	MAERZ & PAUL NUMBER	COMMON NAME	MAERZ & PAUL NUMBER	COMMON NAME
Satsuma orange (Owari)	9-7-L	Deep chrome (orange)	9-1-L	Sulphur (yel- low)	9-2-K	Chrome lemon (yellow)	9-4-L	Sunflower (light orange yellow)
Sweet orange (Hamlin)	9-2-I	Pinard (light orange yellow)	9-1-L	Sulphur (yel- low)	11-1-K	Acacia (dull green yellow)	9-1-J	Deep Martius (yellow)
Tangerine (Dancy)	9-6-K	Golden glow (orange)	9-1-L	Sulphur (yel- low)	9-3-K	Empire (yellow)	9-4-K	Jasmine (yellow)
Key lime	17-2-G	Sea foam (yel- low green)	17-1-J	Martius (light yellow)	17-1-K	Light citronelle (yellow green)	17-1-J	Martius (light yellow)
Grapefruit (McCarthy)	10-1-D	Marguerite (pale yellow)	10-1-F	Light straw (light yellow)	11-1-K	Acacia (dull green yellow)	10-1-F	Light straw (light yellow)

TABLE XIII

EFFECT OF VARYING AMOUNTS OF CITRUS PEEL

ADDED TO 25 CC. JUICE FROM JUICE SACS ONLY	SATSUMA (OWARI) JUICE						
	ORIGINAL COLOR		TIME INTER- VAL	OBSERVED COLOR		ORIGINAL COLOR	
	MAERZ & PAUL NUMBER	COMMON NAME		MAERZ & PAUL NUMBER	COMMON NAME	MAERZ & PAUL NUMBER	COMMON NAME
No treatment (check) ...	9-7-L	Deep chrome (orange)	hr. $\frac{1}{4}$ $\frac{1}{2}$ 3	9-7-L 9-7-L 9-7-L	Deep chrome (orange)	9-6-K	Forsythia (orange)
Distilled orange oil 0.025 cc. ....	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-6-L 9-4-L 9-2-L	Chrome lemon (yellow)	9-6-K	Forsythia (orange)
Distilled orange oil 0.05 cc.	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-4-L 9-3-L 9-1 $\frac{1}{2}$ -L	Chrome lemon (yellow)	9-6-K	Forsythia (orange)
Distilled orange oil 0.25 cc.		Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-2-L 9-1-L 9-1-L	Deep sulphur (yellow)	9-6-K	Forsythia (orange)
Distilled tangerine oil 0.025 cc. ...	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-6-L 9-4-L 9-2-L	Chrome lemon (yellow)	9-6-K	Forsythia (orange)
Distilled tangerine oil 0.05 cc.	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-4-L 9-2 $\frac{1}{2}$ -L 9-1 $\frac{1}{2}$ -L	Chrome lemon (yellow)	9-6-K	Forsythia (orange)
Distilled tangerine oil 0.25 cc.	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-2-L 9-1-L 9-1-L	Deep sulphur (yellow)	9-6-K	Forsythia (orange)
Distilled grapefruit oil 0.025 cc.	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-6-L 9-4-L 9-2-L	Chrome lemon (yellow)	9-6-K	Forsythia (orange)
Distilled grapefruit oil 0.05 cc.	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-3-L 9-2 $\frac{1}{2}$ -L 9-1 $\frac{1}{2}$ -L	Chrome lemon (yellow)	9-6-K	Forsythia (orange)
Distilled grapefruit oil 0.25 cc.	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-2-L 9-1-L 9-1-L	Deep sulphur (yellow)	9-6-K	Forsythia (orange)
Hand-pressed sweet orange oil 0.25 cc.	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-2-L 9-1-L 9-1-L	Deep sulphur (yellow)	9-6-K	Forsythia (orange)
Hand-pressed Satsuma oil 0.25 cc.	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-2-L 9-1-L 9-1-L	Deep sulphur (yellow)	9-6-K	Forsythia (orange)
Hand-pressed tangerine oil 0.25 cc.	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-3-L 9-2-L 9-1-L	Deep sulphur (yellow)	9-6-K	Forsythia (orange)
Hand-pressed lime oil 0.25 cc. ....	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-3-L 9-2-L 9-1-L	Deep sulphur (yellow)	9-6-K	Forsythia (orange)
Orange peel sap less oil 1 cc. ....	9-7-L	Deep chrome (orange)	$\frac{1}{4}$ $\frac{1}{2}$ 3	9-7-L 9-7-L 9-7-L	Deep chrome (orange)	9-6-K	Forsythia (orange)

TABLE XIII

OIL ON COLOR CHANGES IN CITRUS JUICES

TANGERINE (DANCY) JUICE			SWEET ORANGE (HAMLIN) JUICE				
OBSERVED COLOR			ORIGINAL COLOR		OBSERVED COLOR		
TIME INTER- VAL	MAERZ & PAUL NUMBER	COMMON NAME	MAERZ & PAUL NUMBER	COMMON NAME	TIME INTER- VAL	MAERZ & PAUL NUMBER	COMMON NAME
$\frac{1}{2}$	9-6-K	Forsythia (orange)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-2-I	Pinard (light orange yellow)
$\frac{1}{2}$	9-6-K				$\frac{1}{2}$	9-2-I	
3	9-6-K				3	9-2-I	
$\frac{1}{2}$	9-5-K	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-J	Sulphur (yellow)
$\frac{1}{2}$	9-4-K				$\frac{1}{2}$	9-1-K	
3	9-2-L				3	9-1-K	
$\frac{1}{2}$	9-4-K	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-J $\frac{1}{2}$	Sulphur (yellow)
$\frac{1}{2}$	9-3-K $\frac{1}{2}$				$\frac{1}{2}$	9-1-K	
3	9-2-L				3	9-1-K $\frac{1}{2}$	
$\frac{1}{2}$	9-2-L	Deep sulphur (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-K	Deep sulphur (yellow)
$\frac{1}{2}$	9-2-L				$\frac{1}{2}$	9-1-K $\frac{1}{2}$	
3	9-1-I				3	9-1-L	
$\frac{1}{2}$	9-5-K	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-J	Sulphur (yellow)
$\frac{1}{2}$	9-4-K				$\frac{1}{2}$	9-1-J $\frac{1}{2}$	
3	9-2-L				3	9-1-K	
$\frac{1}{2}$	9-4-K $\frac{1}{2}$	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-J $\frac{1}{2}$	Sulphur (yellow)
$\frac{1}{2}$	9-3-K $\frac{1}{2}$				$\frac{1}{2}$	9-1-K	
3	9-2-L				3	9-1-K	
$\frac{1}{2}$	9-3-L	Deep sulphur (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-K	Deep sulphur (yellow)
$\frac{1}{2}$	9-2-L				$\frac{1}{2}$	9-1-K $\frac{1}{2}$	
3	9-1-L				3	9-1-L	
$\frac{1}{2}$	9-5-K	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-J	Sulphur (yellow)
$\frac{1}{2}$	9-3-K				$\frac{1}{2}$	9-1-J $\frac{1}{2}$	
3	9-2-L				3	9-1-K	
$\frac{1}{2}$	9-4-K	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-J $\frac{1}{2}$	Sulphur (yellow)
$\frac{1}{2}$	9-3-L				$\frac{1}{2}$	9-1-K	
3	9-2-L				3	9-1-K $\frac{1}{2}$	
$\frac{1}{2}$	9-3-L	Deep sulphur (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-K	Deep sulphur (yellow)
$\frac{1}{2}$	9-2-L				$\frac{1}{2}$	9-1-L	
3	9-1-L				3	9-1-L	
$\frac{1}{2}$	9-3-K	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-K $\frac{1}{2}$	Deep sulphur (yellow)
$\frac{1}{2}$	9-3-K				$\frac{1}{2}$	9-1-L	
3	9-2-J				3	9-1-L	
$\frac{1}{2}$	9-3 $\frac{1}{2}$ -L	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-K $\frac{1}{2}$	Deep sulphur (yellow)
$\frac{1}{2}$	9-3-L				$\frac{1}{2}$	9-1-L	
3	9-2-L				3	9-1-L	
$\frac{1}{2}$	9-3-L	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-K	Deep sulphur (yellow)
$\frac{1}{2}$	9-3-L				$\frac{1}{2}$	9-1-L	
3	9-2-L				3	9-1-L	
$\frac{1}{2}$	9-3-L	Chrome lemon (yellow)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-1-K	Deep sulphur (yellow)
$\frac{1}{2}$	9-3-L				$\frac{1}{2}$	9-1-L	
3	9-2-L				3	9-1-L	
$\frac{1}{2}$	9-6-K	Forsythia (orange)	9-2-I	Pinard (light orange yellow)	$\frac{1}{2}$	9-2-I	Pinard (light orange yellow)
$\frac{1}{2}$	9-6-K				$\frac{1}{2}$	9-2-I	
3	9-6-K				3	9-2-I	

TABLE XIV  
EFFECT OF VARIOUS OILS ON COLOR CHANGES IN EMPTY JUICE SACS OF CITRUS TYPES

TREATMENT	CLASSIFICATION	SATSUMA ORANGE (OWARI)		TANGERINE (DANCY)		SWEET ORANGE (HAMLIN)	
		ORIGINAL COLOR	OBSERVED COLOR	ORIGINAL COLOR	OBSERVED COLOR	ORIGINAL COLOR	OBSERVED COLOR
Orange oil	Essential oil	9-9-L* Sunkiss (deep orange)	9-4-L Sunflower (light orange yellow)	9-9-J Mikado (orange)	9-5-L Apricot (orange yellow)	9-4-L Sunflower (light orange yellow)	9-1-L Sulphur (yellow)
Clove oil	Essential oil	9-9-L Sunkiss (deep orange)	9-5-K Apricot (orange yellow)	9-9-J Mikado (orange)	9-5-L Apricot (orange yellow)	9-4-L Sunflower (light orange yellow)	9-1-L Sulphur (yellow)
Cedar oil	Essential oil	9-9-L Sunkiss (deep orange)	9-4-L Sunflower (light orange yellow)	9-9-J Mikado (orange)	9-5-L Apricot (orange yellow)	9-4-L Sunflower (light orange yellow)	9-1-L Sulphur (yellow)
Citronella oil	Essential oil	9-9-L Sunkiss (deep orange)	9-4-L Sunflower (light orange yellow)	9-9-J Mikado (orange)	9-5-L Apricot (orange yellow)	9-4-L Sunflower (light orange yellow)	9-1-L Sulphur (yellow)
Turpentine oil	Essential oil	9-9-L Sunkiss (deep orange)	9-4-L Sunflower (light orange yellow)	9-9-J Mikado (orange)	9-5-L Apricot (orange yellow)	9-4-L Sunflower (light orange yellow)	9-1-L Sulphur (yellow)
Canada balsam	Resin	9-9-L Sunkiss (deep orange)	9-4-L Sunflower (light orange yellow)	9-9-J Mikado (orange)	9-5-L Apricot (orange yellow)	9-4-L Sunflower (light orange yellow)	9-1-L Sulphur (yellow)
Avocado oil	Fruit and vegetable oil	9-9-L Sunkiss (deep orange)	9-4-L Sunflower (light orange yellow)	9-9-J Mikado (orange)	9-5-L Apricot (orange yellow)	9-4-L Sunflower (light orange yellow)	9-1-L Sulphur (yellow)
		9-9-L Sunkiss (deep orange)	9-7-L Deep chrome (orange)	9-9-J Mikado (orange)	9-8-K Capucine (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)

TABLE XIV—Continued

TREATMENT	CLASSIFICATION	SATSUMA ORANGE (OWARI)		TANGERINE (DANCY)		SWEET ORANGE (HAMLIN)	
		ORIGINAL COLOR	OBSERVED COLOR	ORIGINAL COLOR	OBSERVED COLOR	ORIGINAL COLOR	OBSERVED COLOR
Pecan oil	Vegetable oil	9-9-L Sunkiss (deep orange)	9-8-L Cadmium (orange)	9-9-J Mikado (orange)	9-8-J Genista (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)
		9-9-L Sunkiss (deep orange)	9-8-L Cadmium (orange)	9-9-J Mikado (orange)	9-8-J Genista (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)
Castor oil	Vegetable oil	9-9-L Sunkiss (deep orange)	9-8-L Cadmium (orange)	9-9-J Mikado (orange)	9-8-J Genista (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)
		9-9-L Sunkiss (deep orange)	9-8-L Cadmium (orange)	9-9-J Mikado (orange)	9-8-J Genista (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)
Linseed oil	Vegetable oil	9-9-L Sunkiss (deep orange)	9-8-L Cadmium (orange)	9-9-J Mikado (orange)	9-8-J Genista (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)
		9-9-L Sunkiss (deep orange)	9-8-L Cadmium (orange)	9-9-J Mikado (orange)	9-8-J Genista (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)
Glycerol	Alcoholic con- stituent of non- volatile fats and oils	9-9-L Sunkiss (deep orange)	9-8-L Cadmium (orange)	9-9-J Mikado (orange)	9-9-J Mikado (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)
		9-9-L Sunkiss (deep orange)	9-8-L Cadmium (orange)	9-9-J Mikado (orange)	9-9-J Mikado (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)
Cod-liver oil	Marine animal oil	9-9-L Sunkiss (deep orange)	9-7-L Deep chrome (orange)	9-9-J Mikado (orange)	9-8-L Cadmium (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)
		9-9-L Sunkiss (deep orange)	9-7-L Deep chrome (orange)	9-9-J Mikado (orange)	9-8-L Cadmium (orange)	9-4-L Sunflower (light orange yellow)	9-4-L Sunflower (light orange yellow)

\* MAEER and PAUL number.

† Common name

figures show also that within these limits the rate of color change was in a marked degree proportional to the amount of oil added.

The experiment was carried a step further in a preliminary attempt to determine the factors involved. The data are shown in table XIV. The following groups of oils and resins and glycerol were added to citrus juice pressed from juice sacs only (Satsuma, tangerine, and sweet orange): (a) essential oils—orange oil (check treatment), clove oil, cedar oil, citronella oil, turpentine; (b) resin—Canada balsam; (c) fruit and vegetable oils—avocado oil, pecan oil, castor oil, linseed oil; (d) cod-liver oil; and (e) alcoholic constituent of non-volatile fats and oils—glycerol. The results were fairly consistent in showing that the essential oils and resins have a similar effect in changing the color of citrus-juice mixtures, as has the check treatment with orange oil, which is also of the same group. The fruit and vegetable oils, cod-liver oil, and the glycerol, had no effect in changing the color except a very slight one in the case of Satsuma orange juice mixture.

In table XV preliminary data are presented showing some changes in color of prepared citrus juices due to the method of extraction and after-treatment, by freezing and cold storage. The characteristic color changes due to small amounts of citrus oil present in the citrus-juice mixture have been discussed. The data presented in table XV are concerned primarily with the effect of freezing and cold storage on the color of the prepared product. In general, it should be noted that in practically all cases there is a slight change in color because of these treatments. The data recorded show that the change was from orange with a medium yellow tone to the same color with a lighter yellow tone. In only a few exceptional cases was there a change in the opposite direction, changes from darker or lighter shades of the same color, or *vice versa*, simultaneous with a change in tone value (see discussion in MAERZ and PAUL 25).

In considering the application of these results, it should be realized that in citrus juices with a naturally deep Satsuma, tangerine, or orange color, any changes to lighter shades of the same color, or to a yellow color, would be a distinct drawback; however, in the case of dull shades of yellow, a change to a brighter yellow may constitute an improvement.

Consideration of the relative amounts of citrus oil required to obtain a given color effect is of importance, not only to those choosing a method of preparation already available, but also to those interested in the design of improved machinery for juice preparation.

As indicated at the beginning of this section, the color factor is secondary to that of taste, and the amount of citrus oil used must in every case be considered from the standpoint of agreeableness in taste of the product.

TABLE XV

EFFECT OF METHOD OF EXTRACTION AND TREATMENT ON COLOR CHANGES IN CITRUS JUICES

VARIETY	DATE	METHOD OF PREPARA- TION	AFTER- TREATMENT	STORAGE PERIOD	COLOR	
					MAERZ & PAUL NUMBER	COMMON NAME
				<i>days</i>		
Sweet orange (Hamlin)	11/20/31	1	Original juice	0	9-1-J	Bright Martius (yellow)
"	"	1	Frozen	5	9-1-I	Martius (yellow)
"	"	2	Original juice	0	9-2-L	Chrome lemon (yellow)
"	"	2	Cold stored	3	9-1-K	Light sulphur (yellow)
"	"	2	Frozen	5	9-1-L	Sulphur (yellow)
"	"	3	Original juice	0	9-3-L	Bright empire (yellow)
"	"	3	Cold stored	3	9-1-K	Light sulphur (yellow)
"	"	3	Frozen	5	9-1½-L	Sulphur (yellow)
"	"	4	Original juice	0	9-4-L	Sunflower (yellow)
"	"	4	Cold stored	3	9-1½-L	Sulphur (yellow)
"	"	4	Frozen	5	9-1½-L	Sulphur (yellow)
Sweet orange (Pineapple)	11/23/31	1	Original juice	0	9-1½-J	Pinard (yellow)
"	"	2	Original juice	0	9-2-L	Chrome lemon (yellow)
"	"	2	Cold stored	2	9-1½-L	Sulphur (yellow)
"	"	2	Frozen	2	9-1½-L	Sulphur (yellow)
"	"	3	Original juice	0	9-2-L	Chrome lemon (yellow)
"	"	3	Cold stored	2	9-1½-L	Sulphur (yellow)
"	"	3	Frozen	2	9-1½-L	Sulphur (yellow)
"	"	4	Original juice	0	9-2-L	Chrome lemon (yellow)
"	"	4	Cold stored	2	9-1½-L	Sulphur (yellow)
"	"	4	Frozen	2	9-1½-L	Sulphur (yellow)
Satsuma (Owari)	11/19/31	1	Original juice	0	9-8-L	Cadmium (deep orange)
"	"	1	Frozen	6	9-6½-L	Golden glow (orange)
"	"	2	Original juice	0	9-9-L	Sunkiss (deep orange)
"	"	2	Cold stored	1	9-8½-L	Cadmium (deep orange)
"	"	2	Cold stored	4	9-8½-L	Cadmium (deep orange)
"	"	2	Cold stored	6	9-8-L	Cadmium (deep orange)
"	"	2	Frozen	6	9-8-L	Cadmium (deep orange)
"	"	3	Original juice	0	9-10-K	Marigold (deep orange)
"	"	3	Cold stored	1	9-9-L	Sunkiss (deep orange)
"	"	3	Cold stored	4	9-8½-L	Cadmium (deep orange)
"	"	3	Cold stored	6	9-8-L	Cadmium (deep orange)
"	"	3	Frozen	6	9-8-L	Cadmium (deep orange)
"	"	4	Original juice	0	9-10-L	Marigold (deep orange)
"	"	4	Cold stored	1	9-9-L	Sunkiss (deep orange)
"	"	4	Cold stored	4	9-8-L	Cadmium (deep orange)
"	"	4	Cold stored	6	9-8-L	Cadmium (deep orange)
"	"	4	Frozen	6	9-8-L	Cadmium (deep orange)
Tangerine (Dancy)	11/23/31	1	Original juice	0	11-3-E	Light maple (dull orange yellow)
"	"	2	Original juice	0	9-7-L	Deep chrome (orange)
"	"	2	Cold stored	4	9-6½-K	Forsythia (orange)
"	"	2	Frozen	4	9-7½-L	Deep chrome (orange)
"	"	3	Original juice	0	9-8-L	Cadmium (deep orange)
"	"	3	Cold stored	4	9-8-K	Capucine (deep orange)
"	"	3	Frozen	4	9-7½-L	Deep chrome (orange)
"	"	4	Original juice	0	9-8-L	Cadmium (deep orange)
"	"	4	Cold stored	4	9-8-K	Capucine (deep orange)
"	"	4	Frozen	4	9-7½-L	Deep chrome (orange)

## CHANGES IN DIRECTION AND RATE OF MOVEMENT OF SUSPENDED PARTICLES

Extracted citrus juices are made up of two distinct phases: one is an almost transparent liquid phase containing mainly dissolved substances, such as sugars, acids, and some colloidal material; the other is a solid phase consisting of small particles of juice sac, locular wall, inner-peel or other tissue in suspension in the liquid phase. The particles in suspension carry the pigment which gives the juice its color, and are closely associated with the characteristic taste of orange juice. Clarified juices do not have this characteristic taste and cannot be substituted for the freshly extracted juice. The larger solid particles begin separating from the liquid phase immediately after extraction. If the juice is allowed to stand long enough undisturbed, there will result an almost purely liquid stratum, which is water clear, and a second stratum or strata of a minimum of liquid and most of the solid particles. In prepared juices this stratification will finally take place, but the methods of preparation and other factors have a pronounced effect upon the *rate* at which it takes place. Other things being equal, the juice showing the slowest rate of stratification is most desirable.

In determining the direction and rate of movement of particles in the juice mixture, the samples were shaken up and poured into 100-cc. graduates, and records of the amount and direction of movement, as well as differences in turbidity in the different parts of the cylinder were taken at 15-min. intervals during the first hour, and at greater intervals for the next 20 hours, and recorded as volume of a stratum.

Only relative differences in turbidity are indicated in the present report. A more accurate nephelometer method (22, 23) is at present under consideration. According to the method used, the turbidity of the sample was observed in the 100-cc. graduated cylinder. The readings were taken primarily on the less turbid upper portion, or on the central portion when some of the suspended particles had risen. The symbols used, and their relative values, are more or less arbitrary: Thus (+++++) represented a condition in which the liquid was practically opaque; increasing degrees of translucency are represented by (+++), (++) , (+), and (+-); when the liquid became almost transparent, so that outlines of objects could be seen through the cylinder, the condition is represented by (+--); increasing degrees of transparency are indicated by (+---), etc.

The prepared citrus-juice mixture, as indicated in the previous discussion, is a buffered biological solution containing in addition extracted solutes and suspended particles derived from the tissues of the fruit during the process of preparation. It was logical, therefore, to look for the causes of changes in the stability of the prepared mixture in the interplay of the factors contained in it and possibly the external environmental conditions. Apparently, therefore, the method of preparation should have a profound

effect upon the total mass in suspension, the direction and rate of its movement up or down, and also the relative turbidity of the liquid mixture. The stage of maturity of the fruit which furnished the juice, and the effect of various subsequent treatments of the juice (such as the containers, gases in contact with the juice, freezing, storage temperature, and length of storage period) are also of importance in this connection.

The exact rôle of the various component parts as they affect the stability of the citrus-juice mixture has not been definitely determined. The constituents possibly affecting the character of the juice mixture may be divided into three classes: (a) substances in true solution, such as sugars, citric acid, proteins, and pectins; (b) substances present in the colloidal state, such as proteins and pectins; (c) matter in mass composed of larger suspended particles.

The first step in the attempt to understand the stability of the suspension was to study the behavior of infusions of the tissues of the fruit in the concentrated condition, also diluted with juice extracted from juice sacs only, and with water. The tissues considered were the outer peel, inner peel and veins, locular walls, central axis, seed coat, and cotyledon and germ. The locular wall, inner peel, and vein infusions gave the most turbid and stable mixtures; the germ and cotyledon infusions also gave turbid and stable infusions. When tissue infusions were diluted in the proportions of 2, 4, and 8 parts of infusion to 100 parts of juice from juice sacs only, or with water, the results paralleled those already indicated, showing that even small amounts of these tissue infusions have a marked effect on the stability of the suspension as measured by the relative turbidity.

As previously stated, the turbidity and color of the juice mixture are due to particles of tissue introduced by the method of extraction, and these tend to separate out from the liquid phase. The direction and rate of movement may be affected by the size and specific gravity of the particles in suspension, or by the possible presence or absence of stabilizing substances such as oils, pectins, and proteins or precipitants (electrolytic or colloidal), or by indirect effects such as might be produced by enzymes. A preliminary report will be given here on the effect of the size of the particles, of citrus oil, and of cold storage and freezing.

The effect of decreasing the size of the particles on the stability of the suspension was studied by using a ball mill and a colloid mill to reduce the size of the particles. In general, the stability of the suspension increases with decrease in size of particles, as indicated by the number of hours the material was ground in a ball mill (table XVI), while the color intensity decreases with decrease in the size of the particles. It will be noted also that the turbidity of the translucent portion is directly correlated with the increase of stability, as indicated by (+ --) turbidity at the end of three hours

TABLE XVI  
EFFECT ON STABILITY OF CITRUS-JUICE SUSPENSION OF DECREASING SIZE OF PARTICLES BY BALL-MILL GRINDING

VARIETY	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	ZONE	VOLUME IN CC. OF ZONE OR ZONES CONTAINING SEPARATED SOLIDS AFTER VARYING PERIODS OF TIME* (HOURS)																
					1	1	1	2	3	4	5	6	8	24							
Tangerine (Dancy) Bartow	11-24-31	2	Untreated	Top				20	18		16	14	18	8							
				Bottom				7	6		20	20	7								
				Turbidity†									+-								
				Top	6	5	5	5	5	3	3	3	0								
"	"	2	Ball mill 1 hour	Bottom { sand†	0	30	26	24	14	14	14	15	7	7							
				tissue	0	0	0	0	5	4	4	5	7	7							
				Turbidity†		++	++	++	++	++	++	++	++								
				Top	No top zone formed																
Tangerine (Dancy) Hawthorne	"	2	Untreated	Bottom { sand†				10	10	8	7		5								
				tissue				0	0	0	1		1								
				Turbidity†				+++	+++	+++	+++	+++	+++								
				Top				57	46	39	35		29								
"	"	2	Ball mill 2 hours	Bottom				0	0	0	8		11								
				Turbidity†				+	+	+	+	+	+	+							
				Top	No top zone formed																
				Bottom { sand†				19	17	14				5							
"	"	2	Ball mill	Bottom { sand†				0	0	0			4								
				tissue				++	++	++				++							
				Turbidity†				++	++	++				++							
				Top										16 hr.							
"	"	2	Ball mill	Bottom { sand†									0								
				tissue										3							
				Turbidity†										4							
				Top										+++							

\* Volumes read in 100-cc. cylinders.

† In grinding a liquid in a ball mill, some sandy or powdery rock material is produced. When juice ground in this way is set up in cylinders, a heavy precipitate, chiefly composed of ground-up rock, comes down quickly and is followed later by settlings that are derived from material in the juice; these zones are separately reported here. The sand zone contained a small proportion of citrus tissue.

‡ Turbidity symbols as indicated in text.

TABLE XVII  
EFFECT OF VACUUMIZATION ON STABILITY OF CITRUS-JUICE SUSPENSION

VARIETY AND SOURCE	DATE PREPARED	METHOD OF PREPARA- TION	AFTER- TREATMENT	ZONE	VOLUME IN CC. OF ZONE OR ZONES CONTAINING SEPARATED SOLIDS AFTER VARYING PERIODS OF TIME* (HOURS)													
					1	$\frac{1}{2}$	$\frac{3}{4}$	1	2	3	4	5	6	8	18	24		
Dancy tangerine (Bartow)	11-24-31	2	Untreated	Top Bottom Turbidity†	20	18	7	6	16	14	18	17	+	---	8			
Dancy tangerine (Hawthorne)	11-24-31	2	"	Top Bottom Turbidity†	57	46	39	35	0	0	0	8	+	---	29			
"	"	2	Juice vacuumized	Top Bottom Turbidity†	0	97	80	58	53	52	+	+	+	---	38			
"	"	2	"	Top Bottom Turbidity†	0	95	92	80	26	+	+	+	+	---	54			

\* Volumes read in 100-cc. cylinders.

† Turbidity symbols as indicated in text.

for Dancy tangerines in untreated material and (+ +) for a similar sample ground in the ball mill for two hours. A second experiment was carried out with juices run through a colloid mill. The results to some extent parallel those secured by the use of the ball mill, although the colloid mill was not as effective in this regard as the ball mill. As was to be expected, the color of the juice mixture changed to a lighter shade with a decrease in the size of the particles. The results obtained by the use of a high-speed soda fountain stirrer were very good, giving a more stable suspension than was obtained by use of the colloid mill.

As has been pointed out earlier, the juices were all subjected to vacuum treatment in order to remove the dissolved gases, a variable factor that would have been brought in if the material had not been treated in this manner. In general, when untreated citrus juices were allowed to stand at room temperature after thorough shaking, they separated into three strata. A small portion of the particles moved upward and a larger portion settled at the bottom, leaving a more or less translucent stratum between (table XVII). When the juices were vacuumized, however, the tendency was for all the particles to move downward, leaving a more or less translucent upper stratum.

The rates of settling during the first few hours in juices prepared by methods 1 and 2 were as a rule similar to the rate in those prepared by method 4, this being the slowest. In the case of method 1, a few larger particles settled out quickly, but the turbidity of the remainder of the juice was so great as to mask this. When the turbidity is greatly reduced for some reason, this layer of coarse particles becomes apparent and the rate of settling would be very rapid if this were taken as the indication of settling. The rate of settling for juices prepared by method 3 is much more rapid than for methods 1, 2, and 4. For method 2 there is little or no settling during the first hour or so after standing. For method 3 there is a rapid settling during the same period, and in method 4 the tendency is again similar to that for method 2. After standing for more than two or three hours, the rate of settling for methods 2 and 4 apparently proceeds at a rate similar to that of method 3; and after twenty hours the amount of settling is approximately the same for methods 2 and 3, but for method 4 the absolute value is higher within the period indicated (table XVIII).

After the juices prepared by the four methods were put in glass containers and vacuumized, two methods of further treatment were employed,—cold storage at 32° F., and freezing at -20° to -25° F. (-28° to -31° C.) with storage in the frozen condition at 0° F. Some of the experimental results concerning the possible effects of the two methods of storage on the stability of the juice mixture are summarized in tables XVIII and XIX.

TABLE XVIII

EFFECT OF METHOD OF EXTRACTION AND AFTER-TREATMENT ON STABILITY OF CITRUS-JUICE SUSPENSION

TYPE OF FRUIT AND VARIETY	DATE PREPARED	METHOD OF PREPARA- TION	AFTER- TREATMENT	STOR- AGE PERIOD	VOLUME IN CC. OF MORE TURBID REGION AND TURBIDITIES* OF LESS TURBID REGION AFTER VARYING PERIODS OF TIME (HOURS)								
					1	1	1	1	2	3	4	24	
Orange (Hamlin)	11/20	1	Original juice	<i>days</i>						8			
"	"	1	Frozen	5	6		6		5	5	5	5	
"	"	2	Original juice		+		++		+	+	+	++	
"	"	2	Cold stored	3	96	94	78	71	61	56	52	48	37
"	"	2	Frozen	5	95		94		81	72	63	37	
"	"	3	Original juice				+		+	+	+	+	
"	"	3	Cold stored	3	98	97	90	84	74	67	63	39	
"	"	3	Frozen	5	74		55		43	40	39	30	
"	"	4	Original juice				+		+	+	+	+	
"	"	4	Cold stored	3	99	98	97	97	97			79	
"	"	4	Frozen	5	98	98	97	97	95	94	92	50	
Satsuma (Owari)	11/19	1	Original juice				12		10	8			
"	"	1	Frozen	6			6		6	6	6	6	
"	"	2	Original juice			97		93	++	++	++	++	
"	"	2	Cold stored	1	98				86	60		44	
"	"	2	Cold stored	4	97	95	90	85	77	70	65		
"	"	2	Cold stored	6		93	91	85	80	70	65	42	
"	"	2	Frozen	6	95		88		68	63	60	40	
"	"	3	Original juice			85		68				33	
"	"	3	Cold stored	1	96				69	52		37	
"	"	3	Cold stored	4	92	86	73	67	59	55	51		
"	"	3	Cold stored	6		80	71	64	61	54	52	37	
"	"	3	Frozen	6	63		46		39	32	30	23	
"	"	4	Original juice			93		75	63				
"	"	4	Cold stored	1	98				83	62		40	
"	"	4	Cold stored	4	98	96	94	91	88	83	78		
"	"	4	Cold stored	6		90	86	83	80	70	66	42	
"	"	4	Frozen	6	72		59		46	42	39	29	
Tangerine (Dancy)	11/23	1	Original juice			95						27	
"	"	2	Original juice					96				63	
"	"	2	Cold stored	4	100	98	98	97	97		89	51	

\* Turbidity symbols as indicated in text.

TABLE XVIII (Continued)

TYPE OF FRUIT AND VARIETY	DATE PREPARED	METHOD OF PREPARA- TION	AFTER- TREATMENT	STOR- AGE PERIOD	VOLUME IN CC. OF MORE TURBID REGION AND TURBIDITIES* OF LESS TURBID REGION AFTER VARYING PERIODS OF TIME (HOURS)								
					1	1	1	1	2	3	4	24	
				days									
Tangerine	11/23	2	Frozen	4	94	84	76	70		46		29	
(Dancy)					+	+	+	+		+		+	
"	"	3	Original juice					98	95	92		61	
"	"	3	Cold stored	4	100	98	97	95	94		72	45	
						+	+	+		+		+	
"	"	3	Frozen	4	94	84	76	70		46		29	
					+	+	+	+		+		+	
"	"	4	Original juice					99	97	94		80	
"	"	4	Cold stored	4	100	99	99	99	99	95		70	
						+	+	+	+	+		+	
"	"	4	Frozen	4	100	97	98	90				59	
						+	+	+				+	
							+						
Lime	7/22	2	Frozen	4 mo.	37	30		25	21	20	19	15	
(Tahiti)					++	++		++	++	++	++	++	
"	"	4	Frozen	4 mo.	40	34		29	25	23	23	15	
					+++	++		++	++	++	++	++	

\* Turbidity symbols as indicated in text.

In table XIX data are presented showing that after four months of storage (juice prepared by method 2 and the rate of freezing varying from 25 minutes to 12 hours) the treatment gave the following results: with the slower rates of freezing, 10 and 12 hours, the settling in general was more rapid than with the quicker rates, 25 to 110 minutes.

The data presented in table XVIII concerning the effect on the rate of settling when juices prepared by the four methods are stored at 32° F., or in the frozen condition at 0°, show with few exceptions that the rate of settling is not markedly affected by these after-treatments.

In certain methods of manufacture, citrus oil is introduced into the juice. An experiment was carried out to determine the effect of citrus oil on the relative stability of the suspension. When a small amount of citrus oil was added to the citrus juice, a portion of the particles were found to rise to the top, as shown in table XX. Other experiments showed that when excessive amounts of citrus oil were added, all of the larger suspended particles in the juice mixture rose to the top.

### Summary

1. The method of localizing in the tissues of the citrus fruit whenever possible the cause or causes of undesirable qualities in the prepared citrus-juice mixture has yielded important results.

TABLE XIX  
EFFECT OF RATE OF FREEZING ON STABILITY OF CITRUS-JUICE SUSPENSION (VALENCIA SWEET ORANGE)

VARIETY	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	STORAGE PERIOD	TIME REQUIRED FOR FREEZING	VOLUME IN CC.* OF ZONE OR ZONES CONTAINING SEPARATED SOLIDS AFTER VARYING PERIODS OF TIME (HOURS) AND TURBIDITY† OF SUPER-NATED LIQUID													
						‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡	‡
Sweet orange (Valencia)	7/24/31	2	Frozen	months 4	25 minutes	100	33	27	21	20	19	17	15	15	15	15	15	15	15
"	"	2	Frozen	4	30 minutes	++	++	+	+	+	+	+	+	+	+	+	+	+	+
"	"	2	Frozen	4	90 minutes	62	48	38	30	27	25	24	22	20	20	20	20	20	20
"	"	2	Frozen	4	110 minutes	++	++	+	+	+	+	+	+	+	+	+	+	+	+
"	"	2	Frozen	4	10 hours	100	44	32	35	23	21	20	20	18	18	18	18	18	18
"	"	2	Frozen	4	12 hours	80	68	53	39	34	31	28	25	23	23	23	23	23	23
						++	++	+	+	+	+	+	+	+	+	+	+	+	+
						60	44	35	27	25	24	21	21	20	20	20	20	20	20
						++	++	+	+	+	+	+	+	+	+	+	+	+	+
						65	49	38	29	27	25	22	22	20	20	20	20	20	20
						++	++	+	+	+	+	+	+	+	+	+	+	+	+

\* Volumes read in 100-cc. cylinder.  
† Turbidity symbols as indicated in text.

**TABLE XX**  
EFFECT OF PRESENCE OF CITRUS OIL ON STABILITY OF CITRUS-JUICE SUSPENSION

VARIETY	DATE PREPARED	METHOD OF PREPARATION	AFTER-TREATMENT	ZONE	VOLUME OF SEPARATED ZONE IN CC. AND TURBIDITY† AFTER VARYING PERIODS OF TIME (HOURS)									
					‡	‡	‡	3‡	6	9	11	24		
Orange (Hamlin)	12-16-31	2 (Vacuumized)	Untreated	Bottom	0	95	84	60	50	45	43	35		
				Top			+++	+++	+++	+++	+++	+++		
		2 (Vacuumized)	1 per cent. citrus oil added	Bottom	90		+++	+++	+++	+++	+++	+++		
				Top*			Breaking	17	15	15	15	10		
				Center*			Curdy	45	40	40	35	34		
				Bottom*				10	10	10	10	10		
				Translucent				+++	+++	+++	+++	+++		
				Separated†				+++	+++	+++	+++	+++		
Tangerine (Dancy)	"	2 (Vacuumized)	Untreated	Bottom	0		85	43	32	29	25	22		
				Top				+++	+++	+++	+++	+++		
				Bottom				+++	+++	+++	+++	+++		
		2 (Vacuumized)	1 per cent. citrus oil added	Top*	0		Curds	35	30	30	30	20		
				Bottom*				10	12	12	12	10		
				Top				+++	+++	+++	+++	+++		
				Center				+++	+++	+++	+++	+++		
				Bottom				+++	+++	+++	+++	+++		

\* When oil was added to orange juice, all of the solids started to rise and then became curdy and separated out to form an upper, a central, and a bottom layer, with two zones of translucent liquid separating them; the volume rather than the exact position of the central zone is shown.

† Since the two translucent zones appeared identical and the turbidity of the zones containing separated solids also appeared identical, the turbidities are given under these two headings.

‡ Turbidity symbols as indicated in text.

2. The cause of the bitter taste which develops in prepared citrus juice on aging has been identified as of glucosidal origin and has been localized primarily in the inner-peel, veins, and locular wall tissues, the degree of its development being a function of fruit maturity, citrus type and variety, method of preparation, and after-treatment.

3. The kind of bitter taste developed for the samples tested was found to depend on the nature of the glucoside contained in the particular type of citrus fruit.

4. The mechanism involved in the development of a bitter taste in the prepared citrus-juice mixture was found to be non-enzymatic in nature.

5. The tendency of prepared citrus juices to develop a bitter taste on aging was found to decrease with maturity of the fruit used. This is in harmony with the known fact that the glucoside content of citrus fruits decreases with maturity.

6. Minimum amounts of citrus-peel oil for the period of time covered by the experiments did not prove objectionable from the standpoint of taste quality.

7. Undesirable taste qualities other than the distinctly bitter taste were encountered under certain conditions. Certain of these must be effectively controlled before a completely satisfactory frozen or cold-stored product can be secured.

8. The causal agent for the observed color changes in prepared citrus juices was traced to the outer peel and identified as citrus oil. When citrus oil was added to the juice mixture the orange or yellow color was changed to brighter shades of the same color, or to yellow, or from yellow to lighter shades of yellow. Somewhat similar results were obtained by neutralizing and by pasteurizing the juice mixture as well as by the addition of some other essential oils and resins.

9. Within the limits of the experiments, the amount and the rate of change in color were in a marked degree proportional to the quantity of citrus oil added.

10. The stability of the suspension is being investigated on the basis of the constituents brought into the juice mixture by the method of manufacture.

11. Reducing the size of the suspended particles by grinding or other treatments improved the stability of the suspension and lightened the color of the product.

12. In prepared citrus juices a portion of the suspended particles rose and another portion settled; following vacuumization all of the suspended particles settled.

13. The method of preparation had a material effect upon the rate of movement of the suspended particles immediately after thorough mixture

with the juice; but after two to four hours' standing the rates of movement of the particles were similar for all methods of preparation used.

14. Preliminary experiments seemed to indicate that rates of freezing had a greater effect upon the stability of the suspension than types of storage. Quick freezing and cold storage of juice did not materially affect the rate of movement of the suspended particles as compared with freshly prepared juice.

15. The presence of small quantities of citrus oil caused a portion of the particles to rise instead of settle and very large amounts caused all particles to rise.

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# SIEVE-TUBE STRUCTURE AND TRANSLOCATION IN THE POTATO

ALDEN S. CRAFTS<sup>1</sup>

(WITH PLATES II-VII AND ONE FIGURE)

## Introduction

The movement of carbohydrates into the potato tuber lends itself to quantitative study. The products of assimilation in the leaves move through the stems and stolon and are condensed to starch within storage cells in the tuber. The stolon may be readily sectioned for measurement; and, knowing the transverse area of its phloem and the rate of development of the tuber, one can calculate the rate of translocation during growth. Photosynthesis is lacking in underground structures, and respiration may be estimated with a fair degree of accuracy.

BIRCH-HIRSCHFELD (3) used a similar method to measure transportation from the leaf of *Phaseolus multiflorus*; and DIXON (10) calculated a rate of 50 cm. per hour for flow of a 10 per cent. sucrose solution through the phloem of a potato stolon during a 100-day growth period. In a previous paper (4) the writer arrived at a somewhat lower rate, 21 cm. per hour, for flow through the total phloem area of the potato stolon during the period of most rapid growth. As respiration losses were not accounted for, this value represents merely the lower limit; actual rates are undoubtedly greater. Many translocation rates have been reported in the literature within the last decade; and, although differing individually, they all approximate this order of magnitude.

Ring experiments, from the early ones of MALPIGHI (18), HALES (12), and KNIGHT (17) to those more recently performed by CURTIS (6, 7, 8), MASON and MASKELL (19, 20), and SCHUMACHER (28), have indicated that organic nutrients synthesized in the leaves move downward through the phloem tissues of the stem. The writer has proposed (4) that the osmotic system described by MÜNCH (22) may provide the force necessary to cause this downward flow; and he has attempted to describe a mechanism (4, 5) which will fit the quantitative data on translocation and at the same time conform to the anatomy of the plant.

Experiments described by SCHUMACHER indicate that the sieve tubes play an important part in the movement of organic materials out of the leaf. As perforation of the sieve plates has probably no essential rôle in longitudinal movement (5), we need some other explanation for the relation of the sieve tube to translocation. A previous paper (5) cited evi-

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dence that the permeability of the sieve tubes in cucurbits increases with maturity. The present work discusses the detailed anatomy of sieve tubes in the potato in relation to translocation, and provides data on rates of flow by which the general theory may be further tested.

### Phloem anatomy

Although ARTSCHWAGER (1, 2) has studied the gross anatomy of the potato, the details of sieve-tube structure have not been described. Because a knowledge of the developmental phases of sieve-tube anatomy must underlie the interpretation of their physiology in relation to translocation, several of these features are included in the present study.

The general vascular structure of the potato resembles that of many herbaceous plants. The bundles are bicollateral. In the stem they tend to remain discrete, being connected laterally by interfascicular cambium; but they fuse more or less in the root and stolon, forming a cylinder of irregular thickness. The phloem is confined to the vascular bundles more strictly than in the cucurbits, but the internal strands follow devious paths and form an anastomosing network in the central region of the stem (1, pl. 34-35). Although anastomosis of the phloem strands is also found in the external phloem, sieve tubes do not occur outside the endodermis or starch sheath; nor do the structural differences noted in the cucurbits (5) appear.

Sieve tubes are perceptible in very early stages in sections of young stolons cut from imbedded material. At the time the sieve-tube mother cell divides to form sieve tube and companion cell, or soon afterwards, the slime body appears in the cytoplasm. ARTSCHWAGER has described and illustrated these bodies (2, pl. 1D, 6, 7), showing one of them in a sieve-tube element, the nucleus of which was still in the metaphase (2, pl. 6B). In the potato these structures differ considerably during their early stages from the slime drops of the cucurbits, only one appearing in each sieve-tube element. In their later development, however, they pass through similar stages, disintegrating as the sieve tube matures.

Plate II, figure 1, shows two of these structures in two successive sieve-tube elements of the young potato stolon. The nuclei are still present, protoplasmic strands have not appeared in the sieve plate, and plasmolysis is apparent in the upper element. Figure 2 shows a slime body in a short connecting element and figure 3 illustrates a later stage. While the slime body and nucleus are present the protoplast seems normal, accumulating neutral red within the vacuole and becoming plasmolyzed in hypertonic solutions. As the element approaches its mature size, however, the nucleus enlarges and disintegrates (fig. 4); the slime body gradually expands, becomes fibrous in structure (plate III, figs. 5, 6), and slowly spreads out

into a tangled mass of gelatinous strands (figs. 7, 8, 9). At this stage, streaming slows down to a plastic flow, and the internal protoplasmic mesh-work described in cucurbits (5) is formed (figs. 9, 10). The tangled mass of disintegrating slime may become attached to these moving protoplasmic strands and presents a peculiar writhing motion.

Small leucoplasts imbedded in the protoplasm, and up to this time practically indistinguishable, begin to accumulate condensed carbohydrate, forming spheres (plate III, fig. 9, plate IV, fig. 16, and plate V, fig. 19) which stain pink or violet with iodine. During the early stages of sieve-tube development, while neutral red is readily accumulated, the vacuoles of these cells are distinct and easily distinguished from the parietal cytoplasmic layer. As the nucleus and slime body disintegrate and the leucoplasts enlarge, the phase boundary between vacuole and cytoplasm apparently breaks down; the plastids migrate from the periphery to the central region of the cell, becoming violently agitated by Brownian movement; and neutral red fails to accumulate within the cell. Slime plug formation, which up to this time has not been noted, is readily induced by means of alcohol and other killing agents. In young tissues (plate III, figs. 6, 8, 10, and plate IV, figs. 11, 12) slime plugs are made up of conglomerate masses of partially disintegrated slime bodies and nuclei, but as disintegration becomes complete they are formed of the more homogeneous suspension that results (plate IV, figs. 13, 14, 15).

The protoplasmic layer of the sieve tube reacts differently to different reagents. The condition depicted in figure 14 results from the use of a killing agent composed of three parts of absolute alcohol to one part of glacial acetic acid. That in figure 15 was induced by 95 per cent. alcohol, which causes a severe shrinking of the protoplasm. The internal strands are present, however, and may be densely stained by the use of iodine and anilin blue (plate IV, figs. 13, 16, and plate V, fig. 17). In figure 13 the slime, which is small in volume, has formed on the surface of the sieve plate a compact layer through which the protoplasmic strands project.

By rapidly killing thin sections of potato stolon tissue in this alcohol-acetic acid mixture, one can avoid contraction and staining of the protoplasm; while the suspension of colloidal material within the cell is coagulated to a flocculent reticulum distributed uniformly throughout the cell (plate V, figs. 18, 19). The protoplasm of these mature sieve tubes shows peculiar properties in fresh sections. All streaming movements have ceased, neutral red no longer accumulates, the cells cannot be plasmolyzed, and anilin blue penetrates readily, staining the callus of the sieve plates (fig. 20) and fragments of colloidal material within the cell. Plate VI, figures 21 and 22 illustrate this stage. The former shows a longitudinal section of phloem of potato stolon that has been allowed to accumulate neutral

red for some time. As soon as this picture was taken, a hypertonic sucrose solution was applied under the cover glass; and, as the cells containing neutral red became plasmolyzed, the photograph shown in figure 22 was made. While the sieve tube in the center of these figures was unaffected, the companion cells and several phloem parenchyma cells were severely plasmolyzed.

Although the protoplasm seems to have passed into an inert condition, it is not actually dead, for treatment with alcohol will often cause a radical change or entire cessation of Brownian movement of the plastids; and killing with dilute eosin solution by the method of SCHUMACHER (28) will cause rapid callusing of the sieve plates and collapse of the elements. Apparently a condition is reached where distinctions between protoplasm and vacuole become less pronounced; the internal strands and parietal layer are simply portions having greater density or viscosity than the intervening regions.

As the sieve tubes become senile, the plates are heavily callused (fig. 20), as are the side wall pits; and in some instances large spherical masses of callus may partially or almost completely fill the cell lumina. Similar callus formations may be induced by the use of dilute eosin solutions (28), and after their development the tubes soon collapse and become obliterated.

The sieve plates of potato develop in the same way as those of cucurbits (5), the chief differences being that the protoplasmic connections are smaller, the callus cylinders are less prominent, and definitive callus formation is less pronounced. The optical effect resulting from the focusing of light by the callus cylinders is very striking in potato sieve plates. Figures 23 and 24 show pictures of the same sieve plate taken at slightly different levels, a water immersion lens and a 12 $\times$  ocular being used. Apparently these plates show an interference phenomenon, the light being blotted out in figure 23 and reinforced in figure 24, for these white spots are much brighter than the original unoccupied field of the microscope.

An oil immersion lens reveals structures similar to those found in cucurbits. The crater-like ends of developing callus cylinders appear in plate VII, figure 25. When stained heavily with anilin blue after being mordanted in iodine, the protoplasmic strands appear as small black dots surrounded by blue callus cylinders (15), which in turn lie in an almost unstained cellulose matrix. The differentiation is difficult to photograph because the differences in color are greater than those in intensity and because one can seldom find plates which are clear of adhering protoplasm and flat enough to show more than a few strands in focus at one time. Figure 26 shows a cup-shaped sieve plate with the edge in focus, giving a longitudinal view of the protoplasmic strands; and figure 27 shows them in transverse view. Although the photographs are not very satisfactory, the

actual preparations are quite convincing and confirm the interpretation that these minute dark-staining cores are the actual protoplasmic strands. As they are only  $0.3\text{--}0.5\ \mu$  in diameter, pores within these strands, if such were present, could probably not be detected by means of the microscope.

The slime bodies in potato sieve tubes have only a short existence. During the major portion of their functioning period, the sieve tubes are devoid of these bodies and of nuclei; the limits of the central vacuoles cannot be detected in fresh sections; a great number of starch-containing plastids are suspended within the confines of the parietal layer; and the protoplasm, by its staining and plasmolytic properties, seems to have lost almost completely its property of semipermeability. The companion cells have nuclei and are rather densely filled with protoplasm, while the phloem parenchyma cells are nucleate and contain starch in increasing quantities as they mature.

### Translocation studies

As detailed anatomical study seems to render untenable the classical theory of mass flow through open pores in the sieve tubes, we must search critically for another mechanism to explain translocation. As in previous work (4, 5), dissection studies have been made on the stems and stolons of the potato.

Phloem exudation is not so pronounced as in the cucurbits, for several reasons. As soon as an incision is made into the phloem, the intercellular spaces of the adjacent parenchyma tissues become filled with sap, acquiring a water-soaked appearance. If another cut is then made near the first, sap will be seen to flow out and wet the surface of the stem with a very thin film that spreads rapidly. These two observations show that the exuded sap has a very low surface tension, differing from that of cucurbits.

If more cuts are made in quick succession near the first one, sap will be seen to accumulate rapidly and run from the wound. If then a cut is made through into the xylem, the sap will be drawn in rapidly and the wounded area will become dry. This shows, as do other experiments, that the phloem exudate for the potato does not coagulate in the same way as cucurbit sap. In addition, one should remember that exudation in the cucurbit stem starts at a rate from three to eleven times that of normal flow (5), while in the potato the stem is apparently less elastic, so that little more than the normal rate is observed.

From measurements of the tubers and stolons of potatoes grown in the field, more reliable rates of translocation may be calculated. The plants were of the Russet Rural variety and were grown by the Division of Vegetable Crops of the College of Agriculture, Cornell University, Ithaca, New York.

TABLE I  
MEASUREMENTS ON TUBERS AND STOLONS OF POTATO

No.	TUBER			STOLON					CELL WALL PHLOEM AREA RATIO per cent.
	FRESH WT. gm.	DRY WT. gm.	DRY WT. AS % OF FRESH WT. per cent.	LENGTH cm.	AREA OF TRANSVERSE SECTION OF			TOTAL PHLOEM cm. <sup>2</sup>	
					STOLON cm. <sup>2</sup>	EXTERNAL PHLOEM cm. <sup>2</sup>	INTERNAL PHLOEM cm. <sup>2</sup> × 10 <sup>-2</sup>		
1	353.0	83.6	23.7	2.0	0.0867	1.98	0.31	2.29	29.7
2	163.0	34.5	21.2	1.8	0.0472	0.63	0.30	0.93	30.2
3	181.5	38.7	21.4	2.3	0.0934	1.28	0.46	1.74	32.7
4	139.5	31.8	22.8	1.7	0.0937	0.93	0.62	1.55	30.7
5	87.0	16.4	18.9	1.7	0.0580	0.83	0.41	1.24	30.2
6	237.6	54.4	22.9	1.4	0.1011	1.03	0.57	1.60	28.6
7	164.5	36.0	21.9	0.5	0.0527	0.63	0.25	0.88	32.4
8	117.2	25.8	22.0	1.3	0.0678	0.85	0.36	1.21	30.1
9	74.2	17.3	23.4	0.8	0.0583	0.60	0.35	0.95	37.8
10	164.3	39.0	23.8	0.9	0.0885	1.11	0.52	1.63	40.1
11	136.0	33.4	24.6	2.2	0.0442	0.55	0.32	0.87	34.2
12	126.6	29.0	22.9	0.8	0.0918	1.11	0.69	1.80	35.6
13	108.2	25.4	23.5	0.2	0.1397	0.90	0.69	1.59	34.5
14	100.5	22.5	22.4	1.3	0.0658	0.97	0.32	1.29	30.7
15	477.2	102.5	21.5	0.8	0.3900	4.21	3.17	7.37	31.1
16	101.8	23.2	22.8	0.3	0.0917	1.52	0.67	2.19	32.5
17	112.5	26.7	23.7	1.8	0.0424	0.47	0.30	0.77	36.6
Total	2,844.6	640.2		21.8	1.6130	19.60	10.31	29.91	
Average	165.0	37.6	22.5	1.3	0.0950	1.15	0.61	1.76	32.5

The seed pieces were planted on May 17, and the tubers were dug on September 22, 1930. Weights and phloem areas are given in table I.

The measurements of sieve-tube elements of both inner and outer phloem appear in table II, together with sieve-tube area: phloem area ratios. The latter are somewhat higher than the ratio given in a previous calculation (4), while the cell walls occupy a somewhat smaller proportion of the total phloem area.

TABLE II  
MEASUREMENTS OF SIEVE-TUBE ELEMENTS IN FIVE POTATO STOLONS

STOLON	EXTERNAL PHLOEM		INTERNAL PHLOEM		SIEVE-TUBE AREA PHLOEM AREA × 100
	NUMBER OF ELEMENTS MEASURED	AVERAGE LENGTH	NUMBER OF ELEMENTS MEASURED	AVERAGE LENGTH	
		<i>mm.</i>		<i>mm.</i>	
1 . . . . .	18	0.114	12	0.123	21.3
2 . . . . .	11	0.117	8	0.109	22.4
3 . . . . .	16	0.159	44	0.108	20.1
4 . . . . .	43	0.107	40	0.088	25.6
5 . . . . .	20	0.096	17	0.082	25.2
Average of all	.	0.106	..	.....	22.9

Measurements were made on sieve plates from these stolons, the protoplasmic strands counted, and the sieve-plate areas surrounding the strands computed, as shown in table III.

The average sieve-plate area surrounding each strand was  $3.04 \times 10^{-8}$  cm.<sup>2</sup> The protoplasmic strands were stained and measured in fully hydrated sections; they averaged  $0.3 \mu$  in diameter. The area of the transverse section of each strand was  $0.0707 \mu^2$ , and the percentage of the sieve-plate area occupied by strands was  $0.0707 \div 3.04 \times 100$ , or 2.3 per cent.

Data on the rate of growth of Russet Rural potatoes under field conditions at Ithaca were provided by Professor E. V. HARDENBURG of the Vegetable Crops Division. The potatoes were planted on June 10, and separate lots harvested on August 25, September 15, and October 17. Table IV shows that between the first two harvests the tubers gained on an average 173.0-90.3, or 82.7 gm. This gain in 21 days gives a rate of gain of 3.94 gm. fresh weight per day. According to the dry weight composition given in table I, the daily increment of dry weight was 0.89 gm.

These tables provide a basis for comparing, in potato, the different theories of translocation. The total phloem area in the stolons of the seventeen tubers, as shown in table I, averaged  $0.0176 \text{ cm.}^2$  In testing the

TABLE III

AREAS, NUMBER OF STRANDS, AND AREA PER STRAND VALUE FOR SIEVE PLATES OF POTATO

SIEVE PLATE	AREA CM. <sup>2</sup> × 10 <sup>-4</sup>	NUMBER OF STRANDS	AREA + NO. OF STRANDS CM. <sup>2</sup> × 10 <sup>-4</sup>
1	3.39	134	2.53
2	1.50	68	2.24
3	2.60	101	2.58
4	2.87	105	2.73
5	2.75	111	2.48
6	6.34	201	3.15
7	4.47	158	2.83
8	2.79	58	4.81
9	2.04	34	6.00
10	2.99	56	5.34
11	3.30	67	4.93
12	3.04	103	2.34
13	3.15	69	4.57
14	3.35	63	5.32
15	2.38	46	5.17
16	1.61	61	2.64
17	1.46	67	2.18
18	2.27	99	2.29
19	1.25	53	2.36
20	4.80	121	3.96
21	4.05	108	3.75
22	5.62	151	3.72
23	3.67	130	2.82
24	3.84	133	2.88
25	1.92	110	1.75
Total	77.45	2507	
Average	3.10	100.3	3.04

protoplasmic streaming hypothesis, it seems best to calculate the movement on a dry weight basis. The daily increment of 0.89 gm. of dry matter would occupy approximately 0.60 cc. and would have to move in the pure state at a linear rate of  $\frac{0.60}{0.0176 \times 24}$ , or 1.42 cm. per hour, through the total phloem area. Only about 10 per cent. of the phloem, however, is occupied by streaming protoplasm, one-half of which is flowing in the proper direction; so that if it were carrying with it organic matter equal to its own volume, and were loading and unloading it with machine-like precision, the rate of movement would still have to be 56.8 cm. per hour in order to deliver the required material. But the maximum rate of streaming observed in phloem parenchyma of the potato was 1.8 cm. per hour, while in mature

TABLE IV  
GROWTH OF POTATO TUBERS

ROW	PLANTED JUNE 10	HARVESTED	TUBERS PER ROW	YIELD PER ROW	WEIGHT PER TUBER
2-7-12 ....	"	Aug. 25	143.5	gm. 12,970	gm. 90.3
3-8-13 ..	"	Sept. 15	141.0	24,390	173.0
1-6-11... ..	"	Oct. 17	157.3	28,900	184.0

sieve tubes no protoplasmic movements occur in sectioned material. Furthermore, since the length of phloem elements is around 0.1 mm., there would be 100 end walls per centimeter, or, in the average conducting system of 30 cm., about 3000 to be crossed by diffusion. If the total gradient in concentration were from 1 molal to 0, then across any one of these membranes the gradient would be  $\frac{M}{3000}$ , an insignificant value so far as its quantitative aspect is concerned. Streaming through the protoplasmic strands of the sieve plate would be equally inadequate, because dry matter alone would have to move at a rate of  $1.42 \div 0.229 \div 0.023$ , or 270 cm. per hour, through the pores occupied by these structures. Even if they were distended by internal pressure, as might be conceivable, they would not suffice, for dry matter alone would have to move 6.2 cm. per hour, or over three times the rate of streaming through the total sieve-tube lumen area. Protoplasmic streaming would seem entirely too slow to act as an effective accelerating or transporting mechanism in the movement of organic nutrients through the potato stolon.

As all of the studies on the structure of sieve plates indicate that the protoplasmic strands traversing them are solid, further testing of the mechanism of mass flow through perforations seems useless. The strands themselves in potato are very slender, being less than  $1 \mu$  in diameter; and any pores which could possibly traverse them would necessarily be so small that an immense pressure would be needed to cause the required rate of flow.

When one considers movement through the intermolecular spaces in the hydrated cellulose of which the phloem walls are composed, the pressure again is obviously of an impossibly high order.

Assuming that these intermolecular spaces occur between concentric cylinders (5), so that the formula as modified to apply to flow between parallel planes might be used, and, inserting a plausible value for the pressure, the value derived for the minimal distance between the planes is  $0.9 \mu$ .

This is from 10 to 100 times too great for pore spaces in a jelly. As there is no justification for assuming that the resistance to flow through cell walls is less than that calculated, the original theory (4) must be modified.

Evidence presented in the previous section indicates that sieve tubes increase in permeability with age; and, in potato, soon reach a condition in which they fail to accumulate neutral red and cannot be plasmolyzed with hypertonic solutions. Repeated trials with sugar beet and cucurbit phloem have given the same results. These findings are contrary to the generally accepted (3, 22) ones of RUHLAND which earlier led the writer to the conclusion that the sieve tubes played no part in the transport of organic nutrients (4). Continued study on this point, however, convinces one that RUHLAND was either dealing with very young sieve tubes or observing other phloem elements.

With the sieve-tube protoplasm completely permeable, the cross walls would present the greatest resistance to mass flow. The pressure necessary to cause movement through the lumina would be almost negligible. If the dry weight increment of the tuber entered in the form of a 10 per cent.

solution, it would move at a linear rate of  $\frac{0.89 \times 9}{0.0176 \times 24} = 19.0$  cm. per hour through a passage equal, in cross-section, to the total phloem. Through the 22.9 per cent. of this area occupied by sieve tubes, it would move at a linear rate of 83.0 cm. per hour, or 0.023 cm. per second, requiring a pressure difference of 0.0035 atmospheres per sq. cm. through the stolon.<sup>2</sup>

A study of the volume relationships of the potato plant indicates that the stolon is a region where the transverse phloem area is constricted. The pressure gradient necessary to cause flow through sieve-tube lumina in the entire plant, therefore, is probably not more than ten times this value, the difference between this and the actual pressure in the phloem being required to overcome the resistance of cross walls.

If the sieve-tube end walls were so arranged as to act as a complete obstruction across the total phloem at frequent intervals, then the area available for longitudinal flow through wall material would be the sum of the sieve-tube lumen area plus the sum of the phloem wall area. An average value for this sum would be, from tables I and II, 55.4 per cent. of the total phloem and the average rate through this area 34.3 cm. per hour. In the potato, however, many of the sieve plates are not transverse and seldom do many of them occur in any one plane. By this arrangement a much

<sup>2</sup> Calculated according to POISEUILLE'S equation,  $P = \frac{8 R_1 n l}{r^4}$  where P = pressure in dynes per sq. cm.;  $R_1$ , linear rate of displacement = 0.023 cm. per sec.; n, viscosity of the solution = 0.012; l, average length of stolon = 1.3 cm.; and r, average sieve-tube radius = 0.0009 cm.  $P = \frac{8 \times 0.023 \times 0.012 \times 1.3}{0.81 \times 10^{-6}} = 3543$  dynes or 0.0035 atmospheres.

greater surface is exposed, reducing even more the actual rate of movement through wall material. This is brought out in text figure 1 *A*, showing the strictly transverse arrangement, and *B*, the situation (greatly foreshortened) as it occurs in potato.

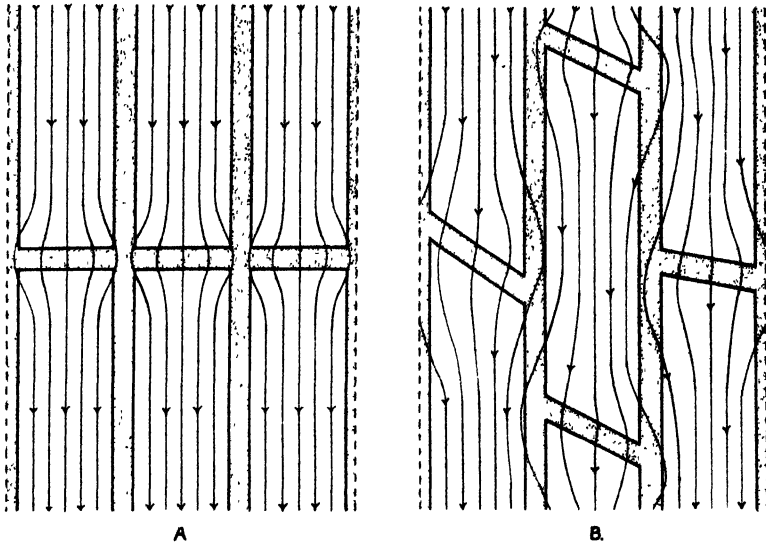


FIG. 1. Diagrammatic representation of flow through potato phloem.

The arrows in these diagrams represent the flow of solution, the distance between them being inversely proportional to the rate of flow. From *B* it can be seen how the actual rate of flow through the wall material may be greatly decreased, reducing the pressure required to maintain movement. By using this reduced rate in the modified formula (5) and substituting the volumes and pressures computed for sap concentrations of 10, 15, and 20 per cent., values have been calculated for the minimal distance between parallel planes which would accommodate this flow. As the stolon is a constricted region, a greater pressure gradient per unit length is required. The values are computed on the assumption that 10, 20, or 50 per cent. of the total available pressure is utilized within this limited region.

These values (table V) show that by assuming ideal conditions, namely, a high concentration in the flowing sap and a high utilization of energy within the limited stolon region, one may arrive at dimensions for capillary spaces which approach the probable values. Respiration has not been considered, and a zero pressure is being assumed at the "sink" or storing parenchyma cell. If, on the other hand, hexose sugars and organic acids of low molecular weight were present in the sap, considerably higher pressures might be expected.

TABLE V

DIMENSIONS OF CAPILLARY SPACES REQUIRED FOR CONDUCTION IN POTATO STOLON;  
CALCULATIONS BASED ON SUCROSE

SOLUTE	CONC.	OSMOTIC PRESSURE AT 25° C.	DIMENSIONS OF CAPILLARY SPACES DISTANCE BETWEEN PARALLEL PLANES		
			PRESSURE UTILIZED		
			10 PER CENT.	20 PER CENT.	50 PER CENT.
<i>per cent.</i>	<i>molal</i>	<i>atm.</i>	$\mu$	$\mu$	$\mu$
10	0.33	8.5	0.066	0.046	0.029
15	0.52	13.5	0.043	0.031	0.019
20	0.73	19.4	0.033	0.023	0.015

The potato stolon is apparently a very efficient conducting organ; and if a mechanism can be found which will satisfactorily explain its functioning, other cases should present no difficulty. Longitudinal movement through structures lacking mature sieve tubes seems to take place along the walls, and dissection experiments indicate that in growing shoots and root tips this movement is not limited to the phloem but spreads throughout the structure. Apparently those tissue layers which, in the mature stem, limit flow to the phloem, have not yet become functional, the epidermis alone serving to check loss by leakage.

MÜNCH (22) has suggested that lateral movement from mesophyll to vascular tissue and from vascular tissue to storage tissues or secondary meristems takes place from living cell to living cell by way of the plasmodesma. In that case, these connecting strands should be abundant in the parenchyma cells of the potato tuber, especially in the vicinity of the phloem strands. Examination of these tissues showed this hypothesis to be correct; and figures 28, 29, and 30 illustrate these structures, stained with gentian violet and iodine. If the plasmodesma are actually wall structures, as JUNGERS (16) has recently suggested, it seems strange that when the wall is swelled by the use of 30 per cent. sulphuric acid to double its original thickness (fig. 31), the plasmodesma should undergo no greater change in length than those treated with only 5 per cent. acid (fig. 32).

Upon entering the tuber, the vascular tissue of the stolon multiplies rapidly in amount and the rate of flow is materially reduced. Lateral diffusion from the strands diminishes this rate even more, longitudinal movement becoming relatively slow.

Measurements were made on two tubers; and the resultant values, given in table VI, aid in the determination of these reduced rates.

TABLE VI  
MEASUREMENTS ON POTATO TUBERS

TUBER NO.	WEIGHT OF TUBER	LENGTH OF TUBER	TRANSVERSE DIAMETERS OF TUBER	VOLUME OF TUBER	AREA OF TRANSVERSE SECTION	NUMBER OF VASCULAR BUNDLES	AREA OF VASCULAR BUNDLES	AREA OF VASCULAR BUNDLES AS PER CENT. OF TOTAL
1	gm. 237.0	cm. 9.4	cm. $7.2 \times 6.1$	cc. 220.3	cm. <sup>2</sup> 33.4	827	cm. <sup>2</sup> 2.07	per cent. 6.2
2	242.0	9.7	$7.5 \times 5.5$	225.0	32.5	806	1.73	5.3
Sum	479.0	19.1	..	445.3	65.9	1633	3.80	...
Average	239.5	9.5	$7.3 \times 5.8$	222.6	32.9	816	1.90	5.8

As the area occupied by phloem in the stolon is roughly proportional to the weight, for these tubers it would be about  $\frac{239.5}{165} \times 0.0176$ , or  $0.0255 \text{ cm.}^2$

Dividing the area occupied by phloem in the vascular bundles of the tuber by this value,  $1.9 \times 2/3 \div 0.0255$ , we find that the phloem expands approximately 50 times within the tuber at its maximum girth. The mean expansion should be about two-thirds this value; and the mean rate of flow within the tuber, if one considers the continual loss by diffusion, should be around 0.285 cm. per hour. The average length of vascular bundle in these tubers is 5.3 cm., and the average time required by the solution to reach its final location within the phloem is  $\frac{5.3}{0.285}$ , or 18.6 hours.

In calculating the rate at which solute diffuses from the phloem to its final destination within the storage cells, the mean distance must be found by computing the radius of the circle whose area is one-half that of the total area of parenchyma surrounding each vascular strand. Correcting for the area of the bundle itself and for the increase in total area due to growth, the mean distance is 0.047 cm., and the mean rate of movement 0.0025 cm. per hour.

As protoplasmic streaming is common within the parenchyma cells of growing potato tubers, it might possibly explain the movement of solutes from end to end of these cells, leaving only the thickness of walls to be traversed by diffusion. According to measurements, the walls occupy about 3.7 per cent. of the distance. Then  $0.047 \times 0.037 = 0.00174 \text{ cm.}$ , the distance to be traveled by diffusion. If streaming accounts for an effective movement of 0.018 cm. per hour, the materials would move 96.3 per cent. of the distance in 2.5 hours; 16.1 hours would remain for diffusion; and  $0.00174 \div 16.1 = 0.00011 \text{ cm.}$ , or  $1.1 \mu$  per hour, the rate of diffusion.

This rate proves to be  $3.01 \times 10^{-8} \text{ cm. per second}$ , or, through unit cross-sectional area,  $3.13 \times 10^{-8} \text{ gm. per second}$ . If the concentration decreased 0.1 molal within the 0.047 cm., the diffusion constant would be  $5.5 \times 10^{-10}$  for movement across the total area, or  $0.11 \times 10^{-7}$  for diffusion through the 5 per cent. occupied by plasmodesma. This value is about 100 times smaller than that for diffusion through water, and closely approximates that obtained by STEWARD (29).

MÜNCH has calculated rates of movement in leaf tissues. From the data on potato (22, p. 87), according to his method, a rate of  $6.25 \mu$  per day for movement within the leaf was obtained. As plasmodesma in the leaf are confined to pits, and as acceleration by streaming would be confined to the parietal layer, the actual rate of movement is probably many times this. If it were as great as 100 times this value, it would be only

625  $\mu$  per day, or 26  $\mu$  per hour. Dividing the mean distance of diffusion, 62.5  $\mu$ , by 26, we find that 2.4 hours would be the average time consumed. Streaming, common again in these tissues, would carry materials 95 per cent. of the way in 0.3 hours; and the remaining distance, 3.125  $\mu$ , divided by the remaining time, 2.1 hours, gives a diffusion rate through the plasmodesma of 1.5  $\mu$  per hour.

As the values obtained by these various calculations all lie within the realm of possibility, apparently this combination of diffusion across walls along plasmodesma, acceleration by streaming within living cells, and mass flow through vascular tissues may satisfactorily explain the conduction of organic nutrients in the potato.

### Discussion

The studies made on potato and on cucurbits indicate that translocation by diffusion (26), protoplasmic streaming (6, 7, 8, 9), young sieve tubes (24), conducting parenchyma cells (11, 26), or perforations through the sieve plates (23, 22) can play little part in phloem exudation or in the rapid transport of carbohydrate into the tuber. While the mechanisms of the first two theories seem too slow and the second two are incompatible with present concepts of the living protoplasmic structures of cells, the last, in spite of its classical position, fails because critical study has not demonstrated the presence of open pores in sieve plates.

The errors of the early workers are not difficult to understand in view of the instruments they used. To HARTIG (13), NÄGELI (23), VON MOHL (21), and SACHS (26), the sieve plates undoubtedly appeared to be truly perforated, and the phenomenon of phloem exudation seemed to confirm this conclusion. One wonders, however, why no one before SCHMIDT (27) pointed out the fallacy of this view, and why his work has aroused so little comment.

SACHS' concept of conducting parenchyma (26), although based almost entirely upon circumstantial evidence and although partially refuted by HEINE (14), is still accepted by HABERLANDT (11) and others.

PRIESTLEY'S idea of movement by streaming through differentiating sieve tubes (24) seems difficult to accept because, in many leaves having practically no secondary tissue, translocation continues long after differentiation has ceased, and because, in such structures as the potato stolon, movement would be restricted to an insignificant fraction of the phloem.

Although the evidence for increasing permeability of the sieve tube with maturity is only fragmentary at present, if subsequent studies, using various methods and many plants, confirm this observation, it should prove ex-

tremely important in relation to rapid translocation. No other mechanism can so well explain the limiting cases exemplified in this study.

### Summary

1. Because the potato is particularly favorable for translocation studies, the anatomy and physiology of phloem tissues in this plant have been investigated.

2. Young sieve tubes of the potato are nucleate and display the characteristics of normal living cells.

3. As the sieve-tube elements mature, the nuclei and slime bodies disintegrate; and the protoplasm apparently changes its organization, becoming more and more permeable.

4. Slime plugs are shown to be artifacts formed by the action of killing agents upon the vacuolar contents; pores within the protoplasmic strands of the sieve plates could not be demonstrated.

5. Phloem exudate from the potato has a low surface tension, does not coagulate rapidly, and seems to emerge at a rate of flow that would account for normal translocation.

6. Measurements indicate that a 10 per cent. sucrose solution would have to flow 19 cm. per hour through a conduit equal in transverse area to the total phloem to provide for tuber formation.

7. The theories of protoplasmic streaming and pressure flow through phloem walls seem inadequate to explain this rate of movement.

8. Sieve-tube lumina apparently afford the most available channels for this movement, the parietal protoplasm offering little resistance. Capillary spaces of from 0.01 to 0.06  $\mu$  would allow movement across end walls under the available pressure.

9. The phloem increases greatly in cross-sectional area within the potato tuber. The rate of flow is correspondingly reduced.

10. Protoplasmic streaming may accelerate lateral movement across non-vascular tissues.

11. Calculations indicate a rate of 1.1  $\mu$  per hour for diffusion along plasmodesma of cross walls in the tuber. The corresponding diffusion rate in the leaf would be 1.5  $\mu$  per hour.

12. Diffusion along plasmodesma of cross walls and acceleration by protoplasmic streaming within non-vascular tissues, combined with pressure flow through permeable sieve tubes and phloem walls within specialized conducting organs, seem most satisfactorily to explain translocation in the potato.

The writer feels deeply indebted to Dr. O. F. CURTIS and other members of the Department of Botany at Cornell University for their aid and interest in this work. He also wishes to thank those members of the Col-

lege of Agriculture at Davis, California, who have helped in the preparation of the manuscript.

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## EXPLANATION OF PLATES

## PLATE II

FIG. 1. Longitudinal section of phloem strand in young potato stolon, showing sieve-tube elements soon after division of sieve-tube mother cell; imbedded material stained with haematoxylin and light green.

FIG. 2. Slime body in short sieve-tube segment of anastomosing internal phloem strands of young potato stolon; same stain as for fig. 1.

FIG. 3. Slime body in developing sieve-tube segment. Note nucleus and densely staining contents of companion cell; same stain as for fig. 1.

FIG. 4. Sieve-tube element in older stolon; nucleus is disintegrating, being enlarged and only lightly stained; staining like fig. 1. All  $\times 750$ .

## PLATE III

FIG. 5. Disintegrating slime body, showing gelatinous strands starting to separate.  $\times 1200$ .

FIG. 6. Disintegrating slime body lodged against sieve plate. Figs. 5 and 6 stained with haematoxylin and light green.  $\times 750$ .

FIG. 7. Disintegrating slime body, showing tangled mass of gelatinous strands lying within the cell lumen.  $\times 900$ .

FIG. 8. Slime body further disintegrated. Strands have become threadlike, and much of the albuminous suspension has accumulated at the sieve plate.  $\times 750$ .

FIG. 9. A few remnants of the slime body may be seen in this well developed sieve tube. Plastids have accumulated considerable carbohydrate material.  $\times 750$ .

FIG. 10. Remnants of the slime body adhering to the internal protoplasmic structure of fully developed sieve tube. Figs. 7-10 are fresh hand-sections with IKI and anilin blue.  $\times 750$ .



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## PLATE IV

FIG. 11. Slime accumulations in young sieve-tube elements of potato stolon. Fragments of slime bodies may be seen within the accumulated masses. Haematoxylin and light green.

FIG. 12. Slime plug in a young, fully differentiated sieve tube. Parietal layer may be seen distinct from the slime.

FIG. 13. Slime accumulated as a thin, compact mass of homogeneous material lodged closely against the sieve plate. Internal protoplasmic strands may be seen projecting through the slime.

FIG. 14. Slime accumulation in a sieve tube killed by applying a mixture of 3 parts of absolute alcohol and 1 part glacial acetic acid to one end of the section. The mass is dense near sieve plate but less and less concentrated toward center of cell. Parietal layer is slightly contracted.

FIG. 15. Dense slime plug in old sieve tube formed by killing with 95 per cent. alcohol. Parietal protoplasm is contracted and surrounds the slime.

FIG. 16. Internal protoplasmic strands in mature sieve tube. Plastids above the plate. Figs. 12-16 stained with anilin blue and iodine. All  $\times 750$ .

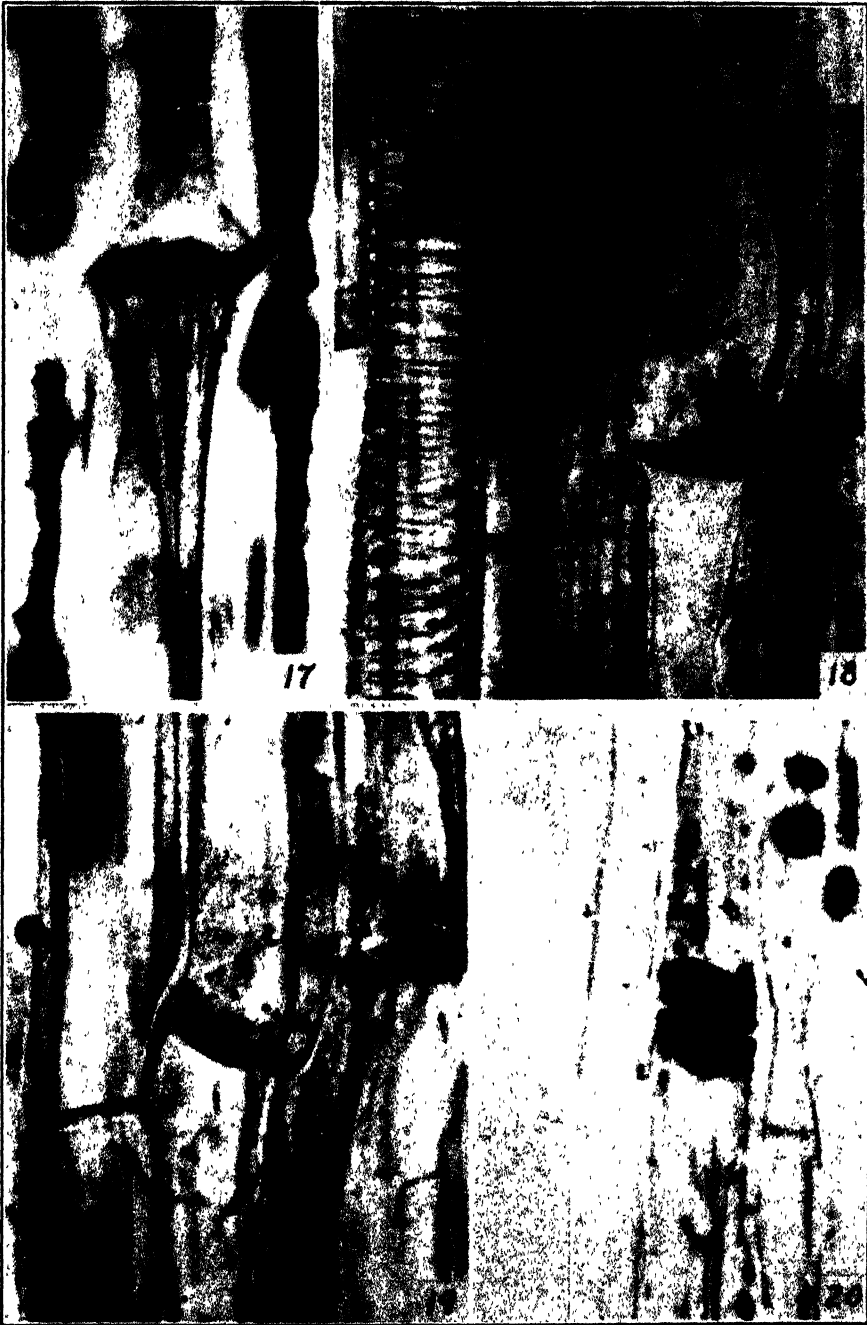
## PLATE V

FIG. 17. Internal protoplasmic strands in mature sieve tube. Under the microscope the threads could be followed directly to the strands of sieve plate. Hand section killed and mordanted in IKI and stained with anilin blue.  $\times 900$ .

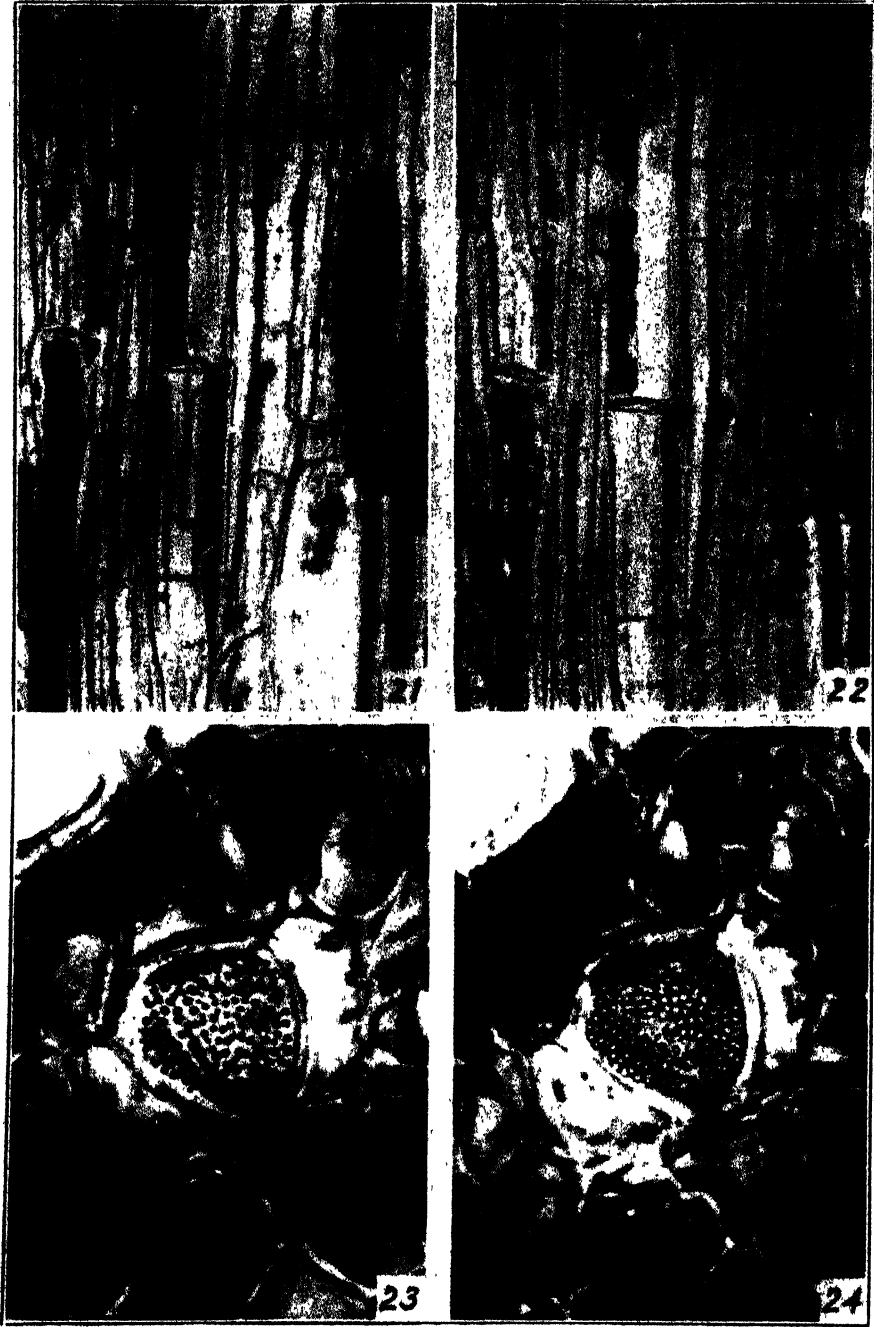
FIG. 18. Sieve tube and spiral vessel of potato stolon. Section killed with absolute alcohol and acetic acid. Parietal and internal protoplasm unstained and not contracted. Sieve-tube lumen contents coagulated to a flocculent reticulum which completely fills the cell. Dark mass at the sieve plate is composed of plastids which were out of focus.  $\times 750$ .

FIG. 19. Another sieve tube in same section as fig. 18, showing plastids more clearly. In living material these plastids are rapidly agitated by Brownian movement. Figs. 18 and 19 stained with anilin blue and iodine.  $\times 750$ .

FIG. 20. Definitive callus on sieve plate stained with anilin blue.  $\times 750$ .



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## PLATE VI

FIG. 21. Longitudinal section of potato stolon, showing sieve tube, companion cells, and phloem parenchyma in living condition after having accumulated neutral red for a short period of time.  $\times 325$ .

FIG. 22. Same section after application of a hypertonic sucrose solution under the cover slip. Sieve tube in center of picture remains unchanged, while companion cells and phloem parenchyma are severely plasmolyzed.  $\times 325$ .

FIGS. 23, 24. Sieve plate of potato stolon stained with iodine and anilin blue as viewed through a water immersion lens. With the focus high (fig. 23), the protoplasmic strands and surrounding callus cylinders appear as black spots. If the focus is slightly lower, the dark spots give way to small bright spots (fig. 24) which are considerably brighter than the unoccupied field of the microscope.  $\times 900$ .

## PLATE VII

FIG. 25. Young sieve plate, showing crater-like ends of callus cylinders which surround the protoplasmic strands. Stained with iodine and dilute anilin blue.  $\times 800$ .

FIG. 26. Cup-shaped sieve plate, showing the narrow protoplasmic strands. Callus cylinders cannot be seen in this photograph because of lack of color differentiation; in the original section they could be seen.  $\times 850$ .

FIG. 27. Transverse section showing sieve plate. Protoplasmic strands surrounded by callus cylinders. The sections in figs. 26 and 27 were stained with a strong solution of anilin blue after being mordanted in a very dilute iodine solution. Protoplasmic strands show black, and callus cylinders blue. A yellow filter was used in taking this photograph.  $\times 850$ .

FIGS. 28, 29. Plasmodesma in potato tuber tissue.  $\times 325$ .

FIG. 30. Plasmodesma of pits, more highly magnified.  $\times 950$ .

FIGS. 31, 32. Thick walls of pith parenchyma of potato, showing plasmodesma in longitudinal view. Figs. 28-32 mordanted in iodine and stained with gentian violet in 5 per cent. sulphuric acid.



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# PLASTID STUDIES IN GENETIC TYPES OF MAIZE: ARGENTIA CHLOROPHYLL

WILLIAM H. EYSTER<sup>1</sup>

(WITH TWELVE FIGURES)

The argentia chlorophyll pattern in maize has played an important part in the genetic studies of the writer since its discovery in 1918. Although a description of this chlorophyll character has already been published (1), it seems desirable, in view of the more recent but erroneous description of ZIRKLE (4), to point out again its principal characteristics. Argentia is a chlorophyll pattern which gives to the leaves a silvery appearance.

Argentia seedlings vary from pure albino to almost normal green in the stage when the first leaf emerges from the coleoptile, as may be seen from the illustration in figure 1. Chlorophyll development begins at the



FIG. 1. Maize seedlings showing argentia chlorophyll.

tips and bases of the leaves and extends longitudinally through the leaf, proceeding most rapidly along the vascular bundles. Consequently, the more or less albinotic leaf becomes transformed into an argentia leaf with green veins and colorless interveinal spaces.

Frequently in the late seedling or early plant stage, the argentia pattern disappears more or less completely, so that argentia plants cannot easily

<sup>1</sup> Fellow of the John Simon Guggenheim Memorial Foundation 1927-1928. Contributions from the Botanical Laboratory of Bucknell University, paper no. 5. Read before the American Society of Plant Physiologists at Cleveland, Ohio, December, 1930.

be distinguished from their normal green sibs. The pattern reappears as the plant approaches maturity, and may vary from a slight trace which is discernible only on the ventral side of the leaf tip to a full expression which extends throughout the entire leaf. A segment of a mature argentia leaf in comparison with a similar segment of a normal green leaf is illustrated in the description previously published (*loc. cit.*).

The nature of the argentia chlorophyll pattern in terms of chloroplastids may be seen from the leaf cross-section shown in figure 2. The chloro-

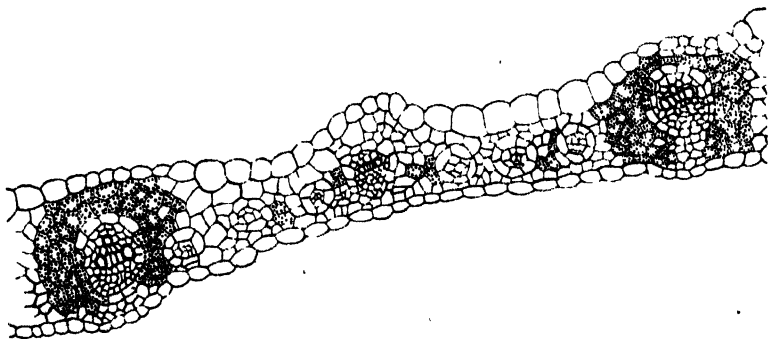


FIG. 2. Cross-section of a part of a leaf of an argentia plant with chlorophyll-bearing cells limited largely to the cells adjacent to the vascular bundles.

plastids in the cells adjacent to the conducting cells of the primary veins are of normal size ( $7\ \mu$  to  $9\ \mu$  in longest diameter) and apparently contain a full complement of the chloroplastid pigments. Occasional cells adjacent to the conducting cells of the secondary veins also have plastids which appear to be normal in size and pigment content. The great majority of the cells between the principal veins, however, contain plastids which are less than  $3\ \mu$  in diameter, and contain only traces of the chloroplastid pigments.

#### **Argentia chlorophyll pattern as affected by environmental factors**

The argentia pattern is highly variable in its expression under different environmental factors, and is especially sensitive to changes in temperature.

The relation between the development of the chloroplastids in argentia and temperature was clearly demonstrated for the first time in a greenhouse planting which extended across a steam trench. The soil directly over the steam trench was quite warm and the homozygous argentia seedlings growing in it were normal green in appearance, while individuals of the same progeny and of the same genetic constitution growing on either side of the trench had the argentia pattern clearly expressed. To check this observation, argentia seedlings of the same genetic constitution were grown under temperatures ranging from  $10$  to  $40^{\circ}\text{C}$ . Under the lowest temperatures,

the seedlings varied from white to cream in color as a result of the almost complete inhibition of chlorophyll development; in temperatures from 15 to 22° C., the argentia pattern was clearly expressed; while in temperatures over 25° C., the seedlings varied from some which had the pattern indistinctly expressed to others which were normal green in appearance. To facilitate the identification of the argentia plants in the genetic studies of maize in which this character is involved, the practice is to keep the greenhouse warm until the seedlings begin to appear through the soil and then to reduce the temperature to that of a semi-cold house.

Under average field conditions, the argentia pattern generally is distinct on the first three or four leaves. The following leaves are usually normal green and the pattern gradually disappears from the first leaves owing to the ultimate development of the chloroplastid pigments in the cells of the interveinal regions, so that the argentia plants can be recognized with difficulty or not at all in the late seedling or early plant stage. Unless the temperature continues abnormally high, the pattern is again expressed more or less clearly in the leaves between the earshoot and the tassel. The pattern is often distinct at the tip when it cannot be recognized in the other parts of the leaf.

A study was made also of the possible effect of light upon the expression of the argentia pattern. Light was found to affect argentia plants in the same manner as it affects green plants. When sufficient light for chlorophyll development is present, the seedlings vary in pattern according to the temperature in which they are growing. When a given temperature is maintained, the pattern cannot be changed markedly by varying the intensity of the light.

### Plastid development and the argentia pattern

Under average or somewhat lower than average temperatures, the first leaves of argentia plants are practically colorless when they are first exerted from the coleoptile. Soon, however, the development of the chloroplastid pigments sets in, beginning at the tip and base of the leaf, and gradually the leaf assumes the argentia pattern, and may finally become uniformly green. In order to determine the nature of these changes, cross-sections of leaves in various stages of development were studied. In the young, colorless leaves, the plastids are small and lack pigments. Chloroplastid development proceeds more rapidly in the cells adjacent to the conductive cells of the vascular bundles than in the other cells of the leaf, and most rapidly in the cells adjacent to the conductive cells of the midrib.

Visible plastids are first apparent in the cells adjacent to the vascular cells which make up the midrib of the leaf, as shown in figure 3. The plastid pigments are first discernible when the plastids are from 2  $\mu$  to 3  $\mu$

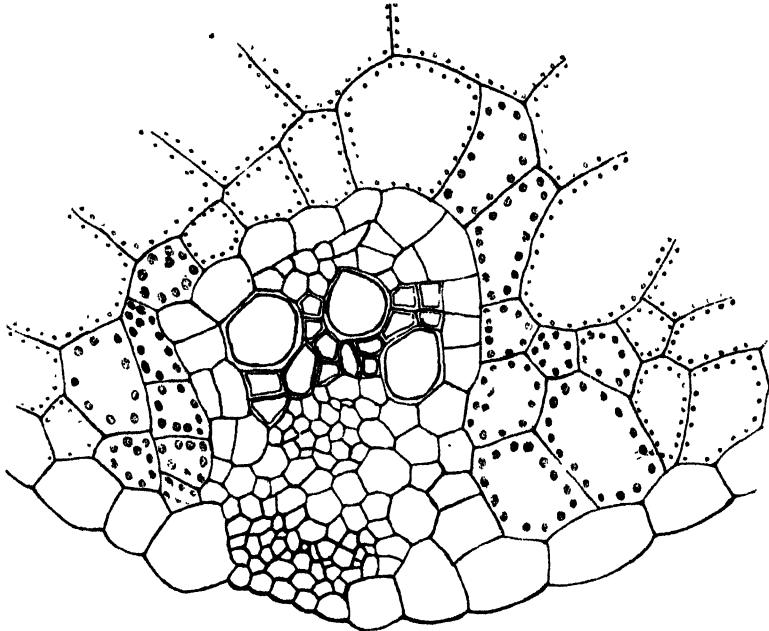


FIG. 3. Cross-section of the midrib showing the first-formed chloroplasts in the leaf.

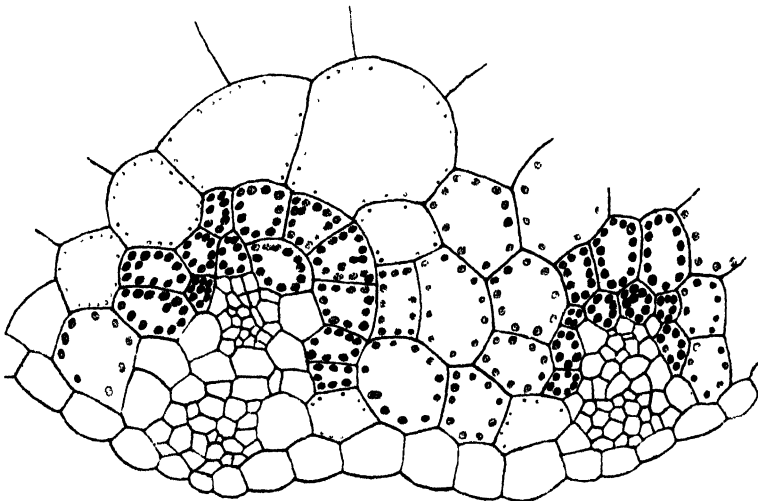


FIG. 4. Cross-section of the midrib and an adjacent vascular bundle showing a later stage in the development of the chloroplasts.

in longest dimension. Plastids become apparent next in the cells adjacent to the conductive cells of the principal vascular bundles on each side of the midrib. The plastids of the cells which ensheath the vascular bundles

have developed sufficient amounts of the chloroplastid pigments to appear green while the plastids in the cells between the bundles are still small and colorless, thus giving to the leaf the *argentina* pattern, which consists in the longitudinal extension of green veins through an otherwise white or cream-colored leaf blade. A somewhat later stage in the development of the chloroplastids in the region of the midrib of the leaf is shown in figure 4. The plastids in the principal and secondary veins are approaching mature size and have enough chlorophyll to appear green while the plastids in the cells between the veins are small and the pigments are just beginning to appear.

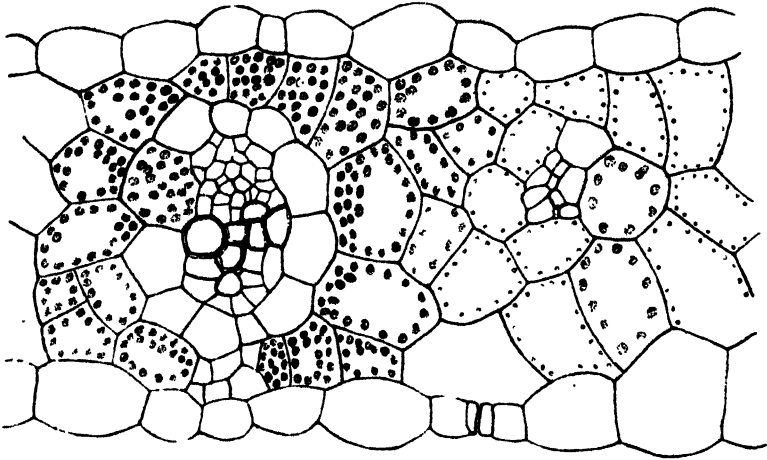


FIG. 5. Section of leaf showing a principal vascular bundle surrounded with chlorophyll-bearing cells while the plastids are just beginning to develop pigments in the cells around the secondary vascular bundle.

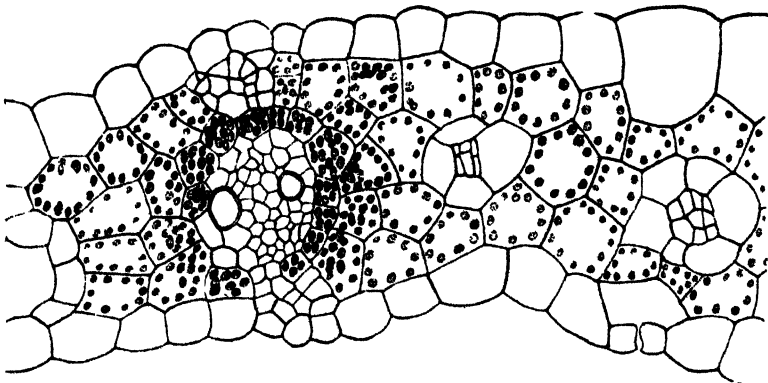


FIG. 6. Cross-section of a part of a leaf with pigments well developed in the cells around the vascular bundles, while the cells in the interveinal regions are beginning to develop the plastid pigments.

Plastid development proceeds more rapidly at the tip than at the base of the leaf. A cross-section of a leaf tip, which is beginning to show the argentia pattern, is illustrated in figure 5. The plastids of the cells adjacent to the conductive cells of the vascular bundles hardly reach mature size and full pigment content before the plastids of the cells in the interveinal regions become pigmented as is indicated in the drawing in figure 6. Soon the plastids of the cells of the interveinal regions attain normal size and pigment content, and the leaf tip becomes uniformly green. A cross-section of a green leaf tip of an argentia leaf is shown in figure 7. This progressive development of the plastids continues until the entire leaf becomes green.

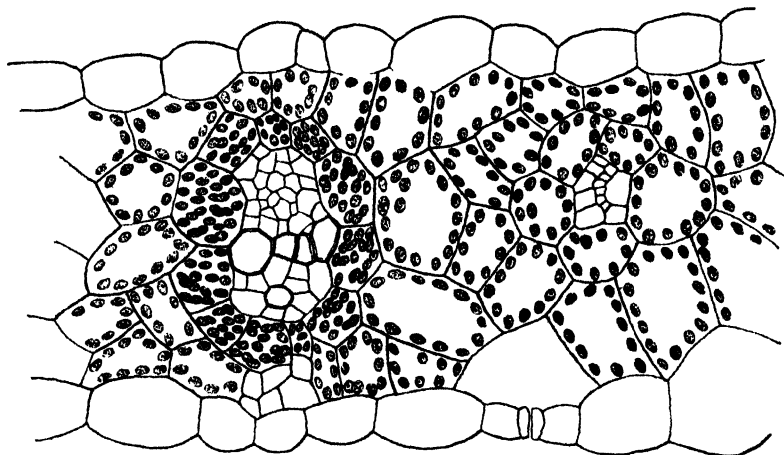


FIG. 7. Cross-section of a leaf tip of an argentia plant which has become uniformly green.

### Inheritance of the argentia chlorophyll pattern

The argentia chlorophyll pattern is inherited as a simple Mendelian recessive character, and is the expression of a gene in the C-Sh-Wx chromosome as shown in a previous publication (*loc. cit.*). Back-cross progenies, including 12,986 individuals, showed a linkage between shrunken endosperm and the argentia chlorophyll pattern with 26.03 per cent. crossing over.

More recently, linkage tests have been made between argentia chlorophyll and waxy endosperm, with the results shown in table I.  $F_1$  plants of the constitution  $\frac{Wx\ ar}{wx\ Ar}$  were back-crossed to double recessives. The progenies from these back-crosses included 2631 plants, and, of these, 286 had the characters in question in new combinations as a result of crossing over. These results indicate a linkage between waxy endosperm and argentia with 10.87 per cent. crossing over.

$F_1$  progenies from the back-cross  $\frac{Wx\ Ar}{wx\ ar} \times \frac{wx\ ar}{wx\ ar}$  are listed in table II.

TABLE I

$$F_1 \text{ PROGENIES FROM THE BACK-CROSS } \frac{Wx\ ar}{wx\ Ar} \times \frac{wx\ ar}{wx\ ar}$$

PEDIGREE	<i>Wx Ar</i>	<i>Wx ar</i>	<i>wx Ar</i>	<i>wx ar</i>	TOTAL
9379-65 .....	21	166	142	19	348
-68 .....	28	128	210	33	467
-74 .....	20	144	210	11	385
-75 .....	36	242	255	25	558
-79 .....	38	218	235	20	511
-86 .....	25	166	161	10	362
Total .....	168	1132	1213	118	2631

These progenies, which involve *wx* and *ar* in the coupling phase, show a linkage between *argentina* and *waxy* endosperm with 10.75 per cent. crossing over. Of the 4075 plants observed, 438 plants had these characters in new combinations as a result of crossing over.

The locus of *wx*, according to EYSTER (1), is at 45.06. The average cross-over value between *wx* and *ar*, as determined from the data in tables I and II, is 10.81. This value may be taken as the map distance between *wx* and *ar* in the chromosome. Since the cross-over value between *sh* and *ar* is greater than that between *sh* and *wx*, it follows that the locus of *ar* is on the opposite side of *wx* from *sh*. Accordingly, the locus of *ar*, as measured from *wx*, is at 55.78. (See fig. 8.)

TABLE II

$$F_1 \text{ PROGENIES FROM THE BACK-CROSS } \frac{Wx\ Ar}{wx\ ar} \times \frac{wx\ ar}{wx\ ar}$$

PEDIGREE	<i>Wx Ar</i>	<i>Wx ar</i>	<i>wx Ar</i>	<i>wx ar</i>	TOTAL
10754-79 × 10769-55	249	34	37	257	577
" -23 × " -2	297	32	35	343	707
" -39 × " -13	313	32	30	271	646
" -54 × " -25	280	44	43	260	627
" -45 × " -4	288	28	25	252	593
" -81 × " -52	153	17	21	136	327
" -20 × " -2	285	31	29	253	598
Total .....	1865	218	220	1772	4075

### Chloroplastid pigments in the *argentina* plants

Analyses of the chloroplastid pigments in *argentina* leaves were made after the methods of WILLSTÄTTER and STOLL (2, 3) with modifications and adaptations as described elsewhere by the writer (in manuscript).

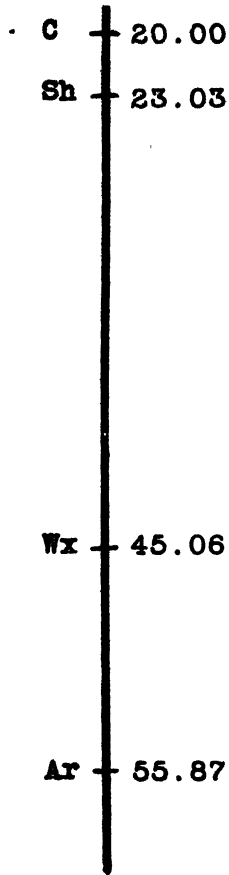


FIG. 8. Chromosome map of a section of the C-Sh-Wx chromosome to show the locus of the Ar gene.

TABLE III

TOTAL CHLOROPHYLL IN GREEN AND ARGENTIA PLANTS

EXPERIMENT	CHLOROPHYLL TYPE	FRESH WEIGHT	TOTAL CHLOROPHYLL	
			TOTAL	PER GRAM
		<i>gm.</i>	<i>mg.</i>	<i>mg.</i>
7a .....	Green	6.430	9.0695	1.410
8a .... ..	Green	7.645	9.1630	1.200
9b ..... ..	Argentia	12.570	9.2395	0.735
10b ..... ..	Argentia	10.300	7.0125	0.681
11b .. ....	Argentia	6.100	7.0452	1.160
12b .... ..	Argentia	13.230	13.8386	1.046

Determinations of total chlorophyll were made from extracts of fresh leaf tissue of (1) *argentina* seedlings, and (2) green plants of the same pedigrees. The results of these determinations are given in table III. The green plants contained from 1.20 to 1.41 mg. of chlorophyll per gram of leaf tissue, while the *argentina* plants contained from 0.681 to 1.16 mg. per gram of leaf tissues. This variation in the total chlorophyll content was due apparently to the differences in temperature under which the seedlings were grown and to differences in the age of seedlings when analyzed. The chlorophyll content of the green plants was, on the average, 1.441 times as much as that of the *argentina* plants. The rather wide variation in chlorophyll content of the *argentina* plants was to be expected because of the variability in the expression of the *argentina* pattern.

Analyses were also made of dry leaf powder of (1) *argentina* plants and (2) green plants of the same genetic strain. (See table IV.) The total

TABLE IV  
TOTAL CHLOROPHYLL IN GREEN vs. ARGENTIA PLANTS

EXPERIMENT	CHLOROPHYLL TYPE	PEDIGREE	DRY WEIGHT	TOTAL CHLOROPHYLL	
				TOTAL	PER GRAM
			<i>gm.</i>	<i>mg.</i>	<i>mg.</i>
1a	Green	10295	2.331	13.1791	5.6538
1b	Argentina	"	2.214	10.4090	4.7014
2a	Green	"	1.982	9.1133	4.5980
3a	Green	10299	1.548	10.4877	6.7750
3b	Argentina	"	1.976	7.6157	3.8541
4a	Green	"	1.548	10.9512	7.0744
5a	Green	"	2.191	8.1639	3.7261
5b	Argentina	"	1.822	8.7473	4.8009

chlorophyll content of the green plants varied from 3.7261 to 7.0744 mg. per gram of dry leaf powder, while that of the *argentina* plants varied from 3.8541 to 4.8009 mg. per gram of leaf powder. A summary comparison of these results indicates that the green plants had, on the average, 1.392 times as much chlorophyll as the *argentina* plants.

Studies were also made of the individual plastid pigments of green and *argentina* plants belonging to the same genetic strain. The individual pigments occur in the green as well as in the *argentina* plants in the general proportions as established by WILLSTÄTTER and his coworkers, as may be seen from the results tabulated in table V. Since the chloroplastids of the *argentina* plants apparently have the pigments in the same proportions as the green plants, it follows that the gene *ar* inhibits the development of all pigments alike. The relation of chlorophyll *a* to chlorophyll *b*, which may

TABLE V

INDIVIDUAL PLASTID PIGMENTS IN GREEN AND ARGENTIA PLANTS, WITH AND WITHOUT ENDOSPERM

EXP.	ENDOSPERM	DRY WEIGHT PER 100 GRAMS FRESH WEIGHT	PIGMENT PER GRAM DRY LEAF TISSUE			
			CHLOROPHYLL		CAROTIN	XANTHO- PHYLL
			a	b		
		gm.	mg.	mg.	mg.	mg.
Ar	Present	8.14	10.8120	2.5671	0.07975	0.12083
ar	"	8.16	8.4891	2.6626	0.14898	0.22199
Ar	Removed	8.20	7.7636	2.4158	0.16740	0.26214
ar	"	8.22	8.2689	2.9579	0.14740	0.22677

be expressed as  $Q\ a/b$ , was found to be 4.21 in the green plants and 3.19 in the argentia plants. This ratio was greatly reduced in plants from which the endosperm had been removed as soon as the first leaf was exerted from the coleoptile. In green plants treated in this manner, the  $Q\ a/b$  was 3.214 while that of argentia plants similarly treated was 2.80. These results would seem to indicate that removing the carbohydrates which are normally available in the endosperm produced a lower value of  $Q\ a/b$  by decreasing the rate of development of chlorophyll *a*.

The relation of carotin to xanthophyll, expressed by  $Q\ c/x$ , was found to be 0.66 in the green plants and 0.67 in the argentia plants. In plants from which the endosperm had been removed when the first leaf was exerted from the coleoptile, the value of  $Q\ c/x$  for green plants was 0.64 and for argentia plants was 0.65. From these comparisons it is concluded that the removal of the endosperm had no effect upon the rate of development of carotin and xanthophyll.

### Chloroplastid pigments and carbon assimilation

A preliminary study of the photosynthetic efficiency of the argentia plants in relation to that of the green plants of the same genetic strain was made by comparing their dry weights. The entire plants were dried in a vacuum desiccator and weighed on a sensitive balance.

The dry weight of young seedlings, based upon a large number of individuals, was determined to be 8.14 per cent. of the fresh weight in green plants, and 8.16 per cent. of the fresh weight in argentia plants. When plants are in the four-leaf stage, the mean dry weight is 6.97 per cent. of the fresh weight in green plants and 6.10 per cent. of the fresh weight in argentia plants. The mean dry weight in pedigree 10335 was 93.5 mg. for green plants and 80.3 mg. for argentia plants.

Some of the plants of pedigree 10337 were dried and weighed at the stage in development when the food reserve in the endosperm had just been exhausted. The mean dry weight of the green plants was 82.253 mg. and that of the *argentina* plants was 76.840 mg. The remainder of the plants were grown until the fourth leaf was full developed, when they were cut off at the level of the ground, dried, and weighed. The mean weight of these older plants was 140 mg. for the green and 74.75 mg. for the *argentina* plants.

A summary comparison of these dry weights shows the green plants to be 1.3684 times as heavy as the *argentina* plants. This weight relationship between green and *argentina* plants is in close agreement with the relation in total chlorophyll content, which was found to be 1.441 as much in green plants as in *argentina* plants in extracts from fresh leaf tissue and 1.392 times as much in green as in *argentina* plants in extracts from dried leaf powder.

#### Removal of endosperm and dry weights in green and *argentina* seedlings

In order to study more closely the relation between chlorophyll content of green and *argentina* seedlings and increases in dry weight, the endosperm was removed from the seedlings of alternate rows in the seed bed at the stage in development when the first leaf was newly exerted from the coleoptile. The seedlings were then kept under conditions favorable for growth until the endosperm in the seedlings from which it had not been removed had been completely digested and absorbed by the growing plants. The young plants were then cut off at the level of the ground, dried in a vacuum desiccator, and weighed individually on a sensitive balance. The results of these weighings are given in table VI, and are compared graphically in figures 9 and 12.

The mean weight of 162 green seedlings was  $68.40 \pm 1.63$  mg. while the mean weight of 209 *argentina* seedlings of the same genetic strain was  $59.96 \pm 1.08$  mg. This difference of  $12.44 \pm 1.96$  mg. presumably was due to the higher chlorophyll content of the green plants. The weight coefficient of *Ar/ar* is 1.22, and is in close agreement with the total chlorophyll coefficient of *Ar/ar*, which was found to be 1.20. This comparison is shown by the curves in figure 9.

The mean weight of 190 green seedlings from which the endosperm had been removed was  $61.05 \pm 1.65$  mg., while the mean weight of 162 green seedlings from which the endosperm had not been removed was  $68.40 \pm 1.63$ . This is a difference of  $7.35 \pm 2.314$  mg., and is doubtless due to the greater chlorophyll content of the seedlings from which the endosperm had not been removed. This comparison is indicated graphically in figure 10.

TABLE VI  
EFFECT OF THE REMOVAL OF ENDOSPERM UPON DRY WEIGHT OF GREEN AND ARGENTIA MAIZE PLANTS

EXPERI- MENT NUMBER	GENETIC TYPE	ENDO- SPERM REMOVED	CLASS CENTERS OF DRY WEIGHT PER PLANT IN MILLIGRAMS														TOTAL	
			5	15	25	35	45	55	65	75	85	95	105	115	125	135		145
13a	Ar	No	1	6	13	14	12	17	24	21	19	5	16	2	7	4	1	162
13b	ar	No	1	9	23	28	37	27	25	23	12	12	8	1	2	1	...	209
14a	Ar	No	1	6	13	14	12	17	24	21	19	5	16	2	7	4	1	162
14b	Ar	Yes	1	19	13	30	24	17	13	23	10	13	8	6	5	5	3	190
15a	ar	No	1	9	23	28	37	27	25	23	12	12	8	1	2	1	...	209
15b	ar	Yes	0	5	24	27	38	30	13	15	13	11	4	2	2	1	1	186
16a	Ar	Yes	1	19	13	30	24	17	13	23	10	13	8	6	5	5	3	190
16b	ar	Yes	0	5	24	27	38	30	13	15	13	11	4	8	2	1	1	186

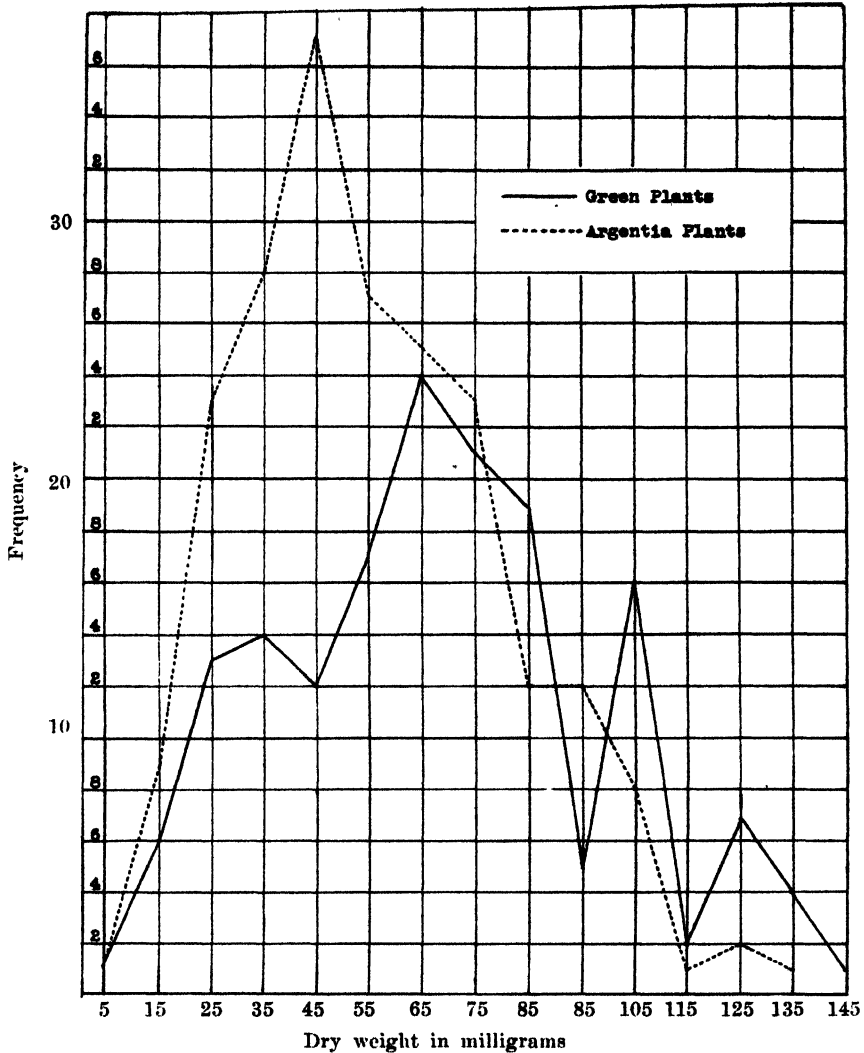


FIG. 9. Variation in dry weight of green and argentia seedlings of the same genetic strain.

A comparison was also made between green and argentia plants from which the endosperm had been removed as already described. The mean weight of 190 green plants was  $61.05 \pm 1.65$  mg., while that of 186 argentia plants was  $55.27 \pm 1.27$  mg. This difference in the mean weight of green and argentia plants is less than three times the probable error of the difference. Since the argentia plants have fewer pigmented plastids than the green plants, these results would seem to show that the chloroplastids of

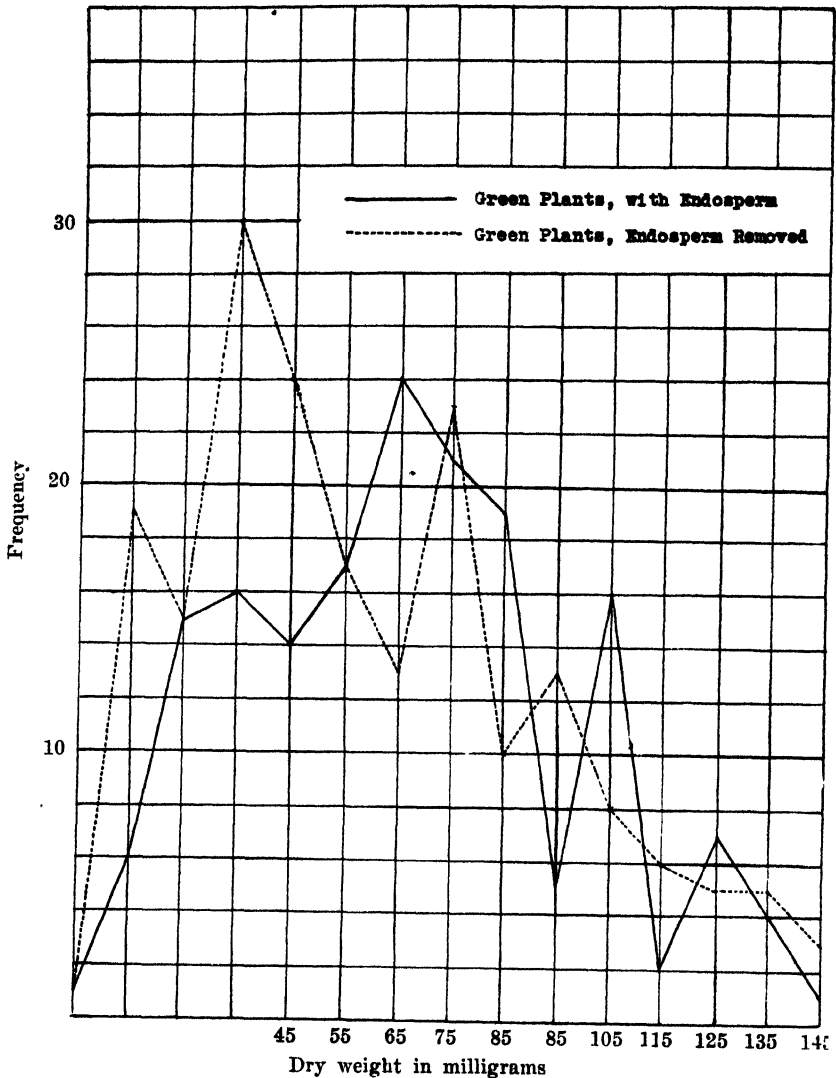


Fig. 10. Comparison in dry weight of green plants with endosperm and green plants from which endosperm had been removed in early seedling stage.

the *argentina* plants have a greater photosynthetic efficiency than those of the green plants from which the endosperm had been removed in the early seedling stage. Although chlorophyll determinations were not made for seedlings from which the endosperm had been removed, it is probable that chlorophyll development is retarded somewhat in the green plants thus treated. A graphic representation of the dry weights of green and *argentina*

plants respectively from which the endosperm had been removed is given in figure 11.

The argentia plants from which the endosperm had been removed increased in dry weight as rapidly as those which possessed endosperm, as shown graphically in figure 12. The mean weight of 209 plants which had an endosperm was  $55.96 \pm 1.08$  mg., while the mean weight of 186 argentina

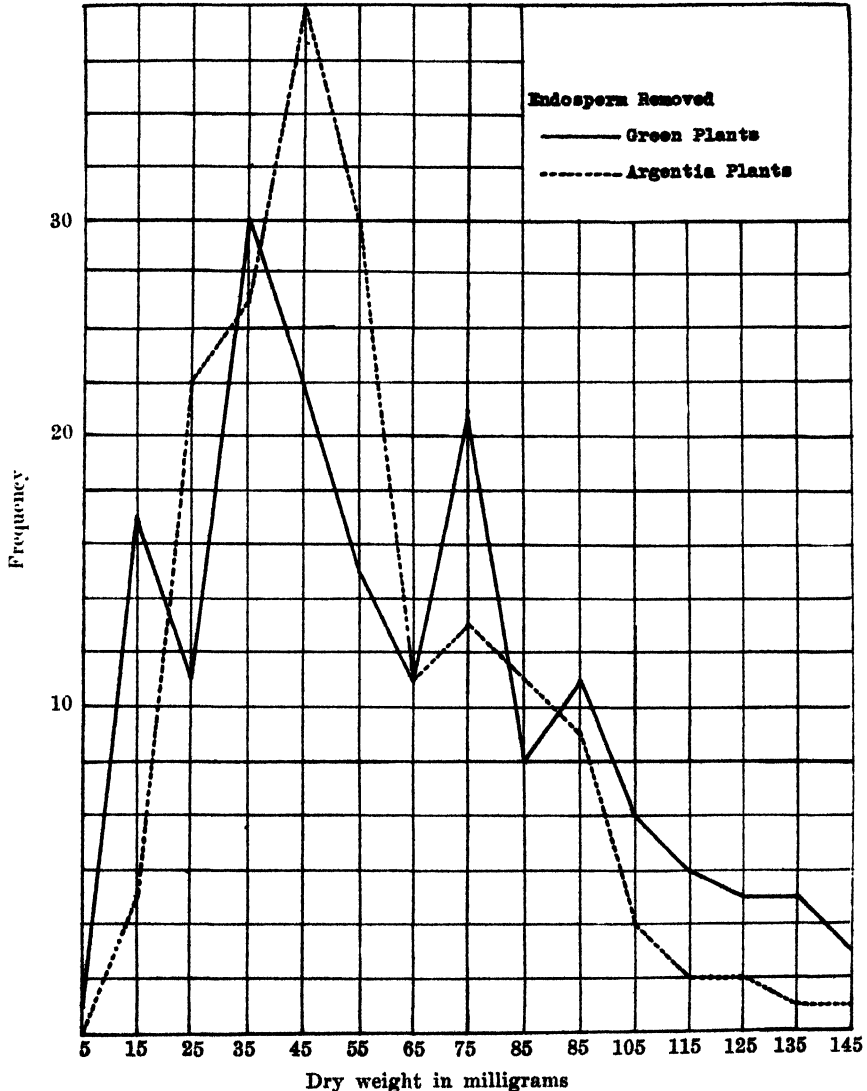


FIG. 11. Comparison in dry weight of green and argentia plants from which endosperm had been removed.

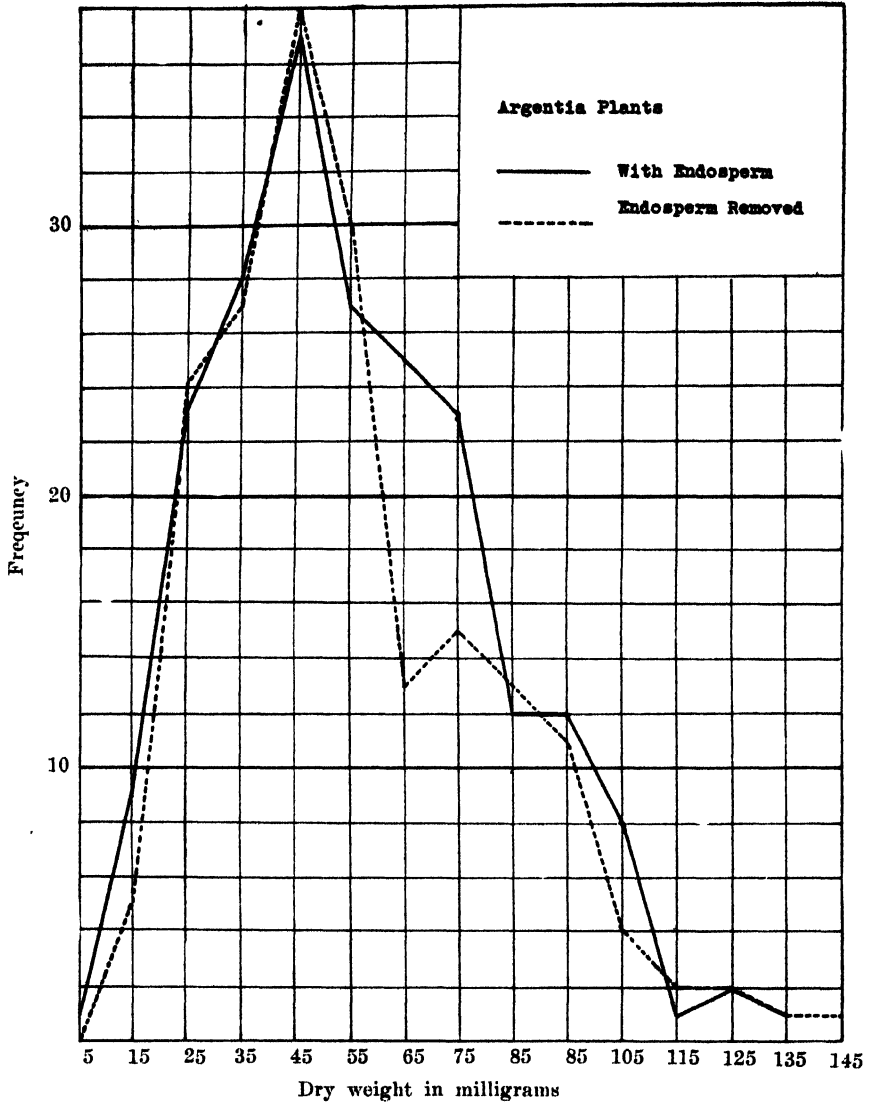


FIG. 12. Comparison in dry weight of argentia seedlings with endosperm and argentia plants from which the endosperm had been removed in early seedling stage.

plants from which the endosperm had been removed was  $55.27 \pm 1.27$  mg. This is a difference of only  $0.69 \pm 1.67$  mg. This result was to be expected, since the argentia plants which lacked endosperm contained as much chlorophyll as those which possessed an endosperm. Why chlorophyll development is not influenced by the removal of the endosperm in the argentia plants,

but is greatly reduced in the green plants when the endosperm is removed in the early stage has not been determined.

### Summary

1. The expression of the *argentina* chlorophyll pattern is influenced by temperature.
2. The *argentina* chlorophyll pattern is the result of the more rapid development of the chloroplastids in the cells which are adjacent to the vascular bundles. The plastids in the other cells of the leaf mesophyll develop pigments tardily or not at all.
3. The *argentina* chlorophyll pattern is inherited as a simple Mendelian recessive character. It is the expression of a gene which has its locus in the C-Sh-Wx chromosome at 10.81 cross-over units to the right of the locus of Wx. The order of the genes in this chromosome is C-Sh-Wx-Ar, as illustrated in figure 8.
4. *Argentina* seedlings have less total chlorophyll than green plants of the same genetic constitution.
5. The chloroplastid pigments occur in *argentina* plants in the same proportions as in the green plants.
6. Chlorophyll content is greatly reduced in green plants by the removal of the endosperm at the stage in development when the first leaf is exerted from the coleoptile.
7. Chlorophyll content in the *argentina* plants is not affected by the removal of the endosperm.

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# EFFECT OF SEVERAL ENVIRONMENTAL FACTORS ON THE HARDENING OF PLANTS<sup>1</sup>

S. T. DEXTER<sup>2</sup>

(WITH FIVE FIGURES)

In several papers (1-3), DEXTER, TOTTINGHAM, and GRABER have outlined a method for determining the injury which plants suffer as a result of freezing. This method involves the determination of the exosmosis of mineral matter from the injured tissues, by measurement of the electrical conductivity of the water in which they have been immersed. Since the method is very sensitive to changes in the hardness of plants, it appeared that the influence of various environmental conditions on hardening could be readily studied. This paper deals with the influence on hardening of light of different day-lengths, and of darkness. The influence of light with and without carbon dioxide (*i.e.*, with and without photosynthesis) was also studied. Several constant temperatures were used, and various combinations of alternating temperatures, both with and without carbon dioxide. Alfalfa, wheat, cabbage, and tomato plants were used for experimental material.

## Experiment 1

On August 25, pots of alfalfa plants of an especially uniform Turkestan strain were transferred from the greenhouse to a large cold room. These plants were grown from seed sown in May, and furnished by Professor L. F. GRABER, of the University of Wisconsin. The pots were one-quart ice cream cartons, paraffined thoroughly. There were generally five plants in each. The plants had never been defoliated. For this experiment, plants under four conditions during hardening were studied: (1) The tops were removed from the plants, and the pots were kept at 0° C. in darkness. (2) The tops were not removed, but the plants were kept in darkness at 0° C. (3) The tops were not removed, and the plants were illuminated for seven hours each day, in a gradually changing temperature which reached a maximum of about 10° C. after four hours of illumination; when the lights went off, the temperature gradually fell to 0° C. Two 1000-watt lamps were used, giving an intensity at the pots of from 900 to 1200 foot-candles, according to the Macbeth illuminometer. Thermograph records were kept. (4) The tops were not removed, but the plants were kept at 0° C. both during illumination and while in the dark. The illumination period was for seven hours, with a slightly greater intensity than in (3).

<sup>1</sup> Contribution from the Hull Botanical Laboratory.

<sup>2</sup> National Research Council fellow in the Biological Sciences.

The temperature at the pots always rose a degree or two when the lights went on, in spite of very thorough stirring of the air with an oscillating 12-inch electric fan.

Each sample tested consisted of the roots of the plants from two pots, trimmed to weigh 5 grams when clean and dried of surplus surface water. The plants were very uniform. The samples were placed in 1×8-inch pyrex test-tubes and frozen in an alcohol-ice slush bath at  $-7^{\circ}\text{C}$ . for four hours. They were thawed by immersion of the tubes in a water-bath at  $2^{\circ}\text{C}$ . for one hour. After thawing, 25 cc. of distilled water at  $2^{\circ}\text{C}$ . were added to each tube in turn. Exosmosis was permitted to continue for 20 hours at  $2^{\circ}\text{C}$ ., when the liquid was drawn off and the electrical conductivity determined at that temperature. Table I shows the results of these determinations after various periods of hardening treatment.

TABLE I

SPECIFIC CONDUCTIVITIES ( $\times 10^4$ ,  $2^{\circ}\text{C}$ .) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF ALFALFA ROOTS FROZEN FOR FOUR HOURS AT  $-7^{\circ}\text{C}$ . INTERVAL OF 20 HOURS ALLOWED FOR EXOSMOSIS AT  $2^{\circ}\text{C}$ .

TREATMENT AND CONDITION	DAYS OF HARDENING TREATMENT					
	0	4	7	14	28	42
1. Tops removed, dark, $0^{\circ}\text{C}$ .	755	845	840	502	551	515
2. Tops not removed, dark, $0^{\circ}\text{C}$ .	755	894	817	478	509	537
3. Tops not removed, 7 hours' light, alternating temperature . . . . .	755	799	573	472	389	408
4. Tops not removed, 7 hours' light, constant temperature . . . . .	755	662	577	468	346	341

Figure 1 shows these results in graphic form. It would appear that the four conditions divide roughly into two classes. The plants in the dark hardened much the same, whether with or without tops; those in the light hardened finally much the same, whether with constant or alternating temperature. At the end of 10 days, since the division into two groups was then becoming evident, two pots were transferred from condition no. 4 into a constant temperature ( $0^{\circ}\text{C}$ .) in the dark, with tops removed. At the end of the experiment, these roots gave a conductivity reading of 551, as against 341 for those continuing in the light. This indicated clearly that hardening had not continued in the dark chamber, although it was well started in the light. Another pair of pots was transferred from condition no. 1 and from condition 2 into the dark but with the alternating tempera-

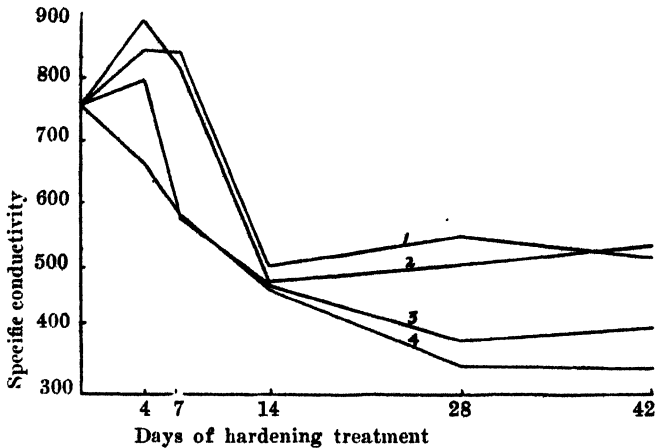


FIG. 1. Curves presenting data of table I in graphic form. The plants in treatment 4 (continuous cold, with light) hardened the most rapidly and the most completely; those in treatment 3 (alternating temperatures, with light) hardened almost as much. Plants in treatments 1 and 2 (in the dark) hardened much less than those in treatments 3 and 4.

ture of condition 3. They gave an average conductivity value of 517 at the end of 42 days. Thus hardening was not notably more efficient with the alternating temperatures in the dark than with the constant temperature in the dark.

On the last date, after 42 days of hardening treatment, the samples were so prepared that buds were left at the crown, in order that recovery in the greenhouse could be noted after freezing and exosmosis. Recovery of the plants showed that the treatments could be readily distinguished. Plants receiving light during hardening made much greater growth and fewer plants died.

#### REPETITIONS OF EXPERIMENT

Experiment 1 was twice repeated, with variations. In each case alfalfa plants in darkness hardened less completely than those in the light. Various day-lengths were used in the hardening chamber at 0° C. and at 10° C. The samples in these experiments were far from uniform, and definite conclusions could not be drawn. In general, hardening was favored by a long period of illumination, if the plants were kept continuously in the cold. In the case of alternating temperatures during hardening treatment, hardening was retarded by the long day at higher temperatures (PELTIER and TYS DAL 5), and elongation of stems was favored. Under this treatment, elongation of stems was especially evident if the plants had been given a short day (7 or 8 hours) previous to such hardening treatment. Figure 2 shows the change that occurred in previously short-day alfalfa plants during



FIG. 2. Condition of alfalfa plants, grown for several months in the greenhouse with a 7 or 8-hour day and then transferred to hardening rooms. The pot on the left received 14 hours of light each day at  $0^{\circ}\text{C}$ ., and was kept in the cold continuously; the pot on the right received 14 hours of light each day at a room temperature of about  $22^{\circ}\text{C}$ . The first pot made no evident growth, while the second grew several inches in 21 days. The first treatment gives good hardening, while the second treatment stimulates top growth and almost prevents hardening.

a treatment in which they were given 14 hours of illumination at  $22^{\circ}\text{C}$ . for 21 days, with 10 hours of each day in the dark at  $0^{\circ}\text{C}$ . While such plants continuously in the cold room ( $0^{\circ}\text{C}$ .), with 14 hours of illumination, hardened from a specific conductivity of 889 at the start to 559 at the end of 21 days, similar plants receiving equal light in the warm room hardened to only 751. The same general relation was found in the case of plants which received light for about 14 hours each day previous to hardening. These plants hardened from 797 to 497 when kept continuously in the cold room with 14 hours of light, while similar plants receiving 14 hours of light in a room at  $22^{\circ}\text{C}$ ., and 10 hours in the dark at  $0^{\circ}\text{C}$ . each day, hardened to only 652. The short-day plants shown had received 7 or 8 hours of daylight in the greenhouse each day for several months previous to hardening treatment; the long-day plants had received 14 or 15 hours of illumination each day for the same period.

In no case did alfalfa plants harden as completely in the dark as in the light. Alternating temperatures were no more effective than continuous ones in any case studied. If the illumination tended to favor much extension of parts, hardening was retarded or prevented. Hardening was more

thorough at 0° C. than at 10° C. Although these plants were in general very well stored with starch and other reserve foods, as shown by micro-inspection, hardening was always more complete in the light, when photosynthesis could furnish a continually replenished supply of carbohydrates for the processes of the plants.

### Experiment 2

In this experiment, Minhardi winter wheat was grown in perforated soil-temperature cans (6 × 9-inch cylinders) in soil which was thoroughly sifted and mixed. The seed was sown on November 25, the plants were thinned to 15 plants per can, and, from the first, were exposed to two day-lengths in the greenhouse, at about 62° F. One set received ordinary daylight (reinforced with Mazda lamps on especially dark days) for 8 hours each day; the other set received the full day-length, with lights (four 200-watt, about 2 feet away) extending the illumination period until 10 P. M., which gave approximately a 14-hour day. At an age of six weeks, the short-day plants were much smaller and somewhat greener than the long-day plants. Fifteen short-day plants weighed, entire, 3.78 grams; 15 long-day plants, 7.0 grams. The short-day plants (leaves) had a percentage dry matter of 14.0; the long day, 13.0. The percentage ash (wet-weight basis) was slightly higher in the short-day plants than in the long-day plants, 2.22 as against 1.96. The electrical conductivity of the ash solution, after converting the basic oxides to carbonates and made to volume on a wet-weight basis, was about 2 per cent. higher in the short-day plants.

These plants were taken to the hardening chamber on January 6 (six weeks old), where several pots from each set (long- and short-day) were put to harden in the following ways: (1) in the dark; (2) with 7 hours of light each day; (3) with 15 hours of light each day. All were at 0° C. Two 1000-watt lamps furnished the light, as in the preceding experiment. Precaution was taken to avoid injury to the plants by lowering the temperature of the room gradually, taking about 6 or 7 hours. Very little wilting was noted. The samples were prepared by removing the roots and leaf blades, and washing and drying the remaining crowns. Duplicate samples weighed 0.9 gram each; they were frozen for 2 hours at -10° C. in an alcohol-slush bath and thawed for 30 minutes in the water-bath at 2° C. Fifteen cc. of distilled water (2° C.) was then added to each tube. Exosmosis went on for 16 hours at 2° C., when conductivity measurements were made. Table II shows the conductivity values obtained in this experiment after the durations of hardening treatment indicated.

Table II shows consistent results throughout. The plants, whether long- or short-day in the greenhouse, hardened little if at all in the dark; they hardened materially in 7 hours of light, and still more when given 15 hours

TABLE II

SPECIFIC CONDUCTIVITIES ( $\times 10^6$ ,  $2^\circ \text{C.}$ ) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR TWO HOURS AT  $-10^\circ \text{C.}$   
INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT  $2^\circ \text{C.}$

DAY-LENGTH IN GREENHOUSE	HOURS OF LIGHT IN HARDENING ROOM	DAYS OF HARDENING TREATMENT			
		0	3	7	21
Short	0	382	393	384	386
	7	382	262	222	113
	14	382	218	174	89
Long	0	447	446	431	387
	7	447	315	317	171
	14	447	307	224	138

of light each day. In the dark, the short-day plants hardened not at all; the long-day plants hardened very slowly, but perceptibly.

Since it was clear at the end of the first week that but little hardening was going on in the plants in the dark, it seemed desirable to determine the result when plants were given light, but not carbon dioxide. Two pots were taken from the long-day (greenhouse) set which had been hardening in the dark for 10 days. One pot was put under a bell-jar which was sealed with sodium hydroxide solution on the bottom; free access to air was provided through a soda-lime tube at the top. Paper soaked in sodium hydroxide solution was stuck to a portion of the side wall of the bell-jar to take up any carbon dioxide set free in respiration. The other pot was similarly covered with a bell-jar which was sealed with water at the bottom. To this latter jar a carbon dioxide generator was connected each morning. Phosphoric acid was placed in the generator flask, and 100 cc. of M/50 sodium carbonate were allowed to flow slowly, through a capillary tube, into the acid. This slowly set free the carbon dioxide for a period of about three hours, it flowing through the delivery tube into the bell-jar. In this way, between 40 and 50 cc. of carbon dioxide gas were supplied to the pot each day. The bell-jars were placed side by side at  $0^\circ \text{C.}$ , and were illuminated for 15 hours each day. This hardening period lasted for 12 days, when samples were prepared and treated as usual. Table III shows the results of determinations of electrical conductivity with duplicate samples from three pots, of which one was in the dark during this period, the second in the light without carbon dioxide, and the third in the light with carbon dioxide.

TABLE III

SPECIFIC CONDUCTIVITIES ( $\times 10^6$ ,  $2^\circ \text{C.}$ ) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR TWO HOURS AT  $-10^\circ \text{C.}$   
INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT  $2^\circ \text{C.}$

CONDITION DURING HARDENING	SPECIFIC CONDUCTIVITY
No illumination, $0^\circ \text{C.}$ . . . . .	387
Illuminated 15 hours each day, $0^\circ \text{C.}$ , no $\text{CO}_2$ . . . . .	394
Illuminated 15 hours each day, $0^\circ \text{C.}$ , given $\text{CO}_2$ . . . . .	222

The figures in table III show that hardening proceeded almost identically in the cases of plants in the dark and those illuminated, but without carbon dioxide in the surrounding atmosphere. The plants which received carbon dioxide hardened in the usual way. This seems to indicate that hardening is definitely associated, in these rather succulent tissues, with photosynthesis, and that the influence of light at this temperature may be largely due to carbohydrate synthesis.

At the end of the hardening treatment, two pots of short-day (greenhouse) plants, one from the dark, the other from the long-period illumination treatment, were harvested for the determination of dry matter. The hardened plants (from the light) were much higher in dry matter (20.8 as compared with 13.0 per cent.) than those which had not hardened (in the dark). Curiously, however, the total amount of dry matter in the hardened plants had not increased in any such proportion. There seemed, therefore, to have been a dehydration process going on in the hardening plants, in the light, which increased the percentage of dry matter by loss of water as well as by gain in carbohydrates. Further study may determine whether or not this is actually the case.

#### REPETITIONS OF EXPERIMENT

Experiment 2 was repeated, with variations, with plants of three varieties of winter wheat grown out-of-doors until five weeks of age (September 23 to October 27). When these plants were given hardening treatment at  $0^\circ \text{C.}$  in the dark, with 7 hours and with 15 hours of illumination each day, for a period of 28 days, hardening was least complete in each case in darkness, more complete with 7 hours, and most complete with 15 hours of illumination. The average (for the three varieties) conductivity value at the beginning of hardening treatment was 884; in the dark this value was 398 at the end of the hardening period. With 7 hours of illumination each day the value was 292; and with 15 hours, 237. These plants, which were much more sturdy and higher in dry matter than the plants grown in the

greenhouse, hardened considerably in the dark, whereas the succulent greenhouse plants did not. The alfalfa plants which were well stored with reserve foods (experiment 1) also hardened somewhat in the dark. In each case, however, hardening was more complete in the light.

The plants of the three varieties of wheat were also given 7 and 15 hours of illumination each day at 10° C., for 28 days. These plants, while hardening slightly at first, became very chlorotic and more tender as the experiment proceeded. As in the case of the alfalfa plants, those receiving the longer day at this higher temperature were more tender than those receiving the 7-hour day.

### Experiment 3

In this experiment, the effect of the presence or absence of carbon dioxide in the air was studied at different temperatures and with four species of plants. Minhardi wheat, hardy alfalfa, cabbage, and tomato plants were used. The wheat, cabbage, and tomato plants were grown in sand culture with nutrient solutions, and were five weeks old at the beginning of the hardening treatment. The plants were very uniform in appearance. The alfalfa plants were nine weeks old when hardening treatment began. They were grown in soil and were somewhat irregular in appearance. The wheat received a short day (8 hours) in the greenhouse, while the other plants received a long day (about 14 hours). The photographs show the size of the plants. There were 30 wheat plants, approximately 30 alfalfa plants, 8 tomato plants, and 8 cabbage plants, respectively, in each can. Samples were prepared as follows. The wheat samples were prepared as in experiment 2, using duplicate 1-gram samples. The alfalfa root samples were prepared by removing the leaves at the crown, washing in distilled water, and drying the surface water. A single 0.5-gram sample (about 25 roots) was used. The tomato and cabbage samples were prepared by removing the roots, and washing the tops and drying between towels. Duplicate 3-gram samples of the tops of each were used. The wheat and alfalfa samples were placed in test-tubes and were frozen in an alcohol-ice slush at -8° C. for 2 hours; they were thawed in the water-bath at 2° C. for 30 minutes. To each tube in turn, 10 cc. of distilled water at 2° C. were added. Exosmosis continued for 16 hours at 2° C., when the electrical conductivity was measured at that temperature. The cabbage and tomato samples were placed in 1×8-inch test-tubes, frozen at -5° C. for 4 hours, thawed as indicated, and 25 cc. of distilled water at 2° C. added to each tube. Conductivity measurements were made after 14 hours of exosmosis. There is generally considerable super-cooling without freezing when plant tissues are subjected to a temperature no colder than -5° C. This experimental difficulty was met with regularly when this temperature was used. If,

however, the tissues were moved about in the tube with a clean glass rod after they had been thoroughly chilled, or if the tube was sharply rapped against a solid body, freezing generally occurred rather promptly. In some cases, especially when the plants were partially hardened, it was necessary to start crystallization by placing the tubes for a moment or two in a colder bath ( $-7$  or  $-8^{\circ}$  C.), after which they were replaced in the  $-5^{\circ}$  C. bath where freezing always continued without difficulty.

The plants in this experiment were hardened in the following way. Two glass-walled chambers, about 8 cubic feet each, were placed in the cold room at  $0^{\circ}$  C. Into each chamber a tube of air was led from a compressed air line. In the case of one chamber, the air was merely dehydrated (with sulphuric acid and glass wool); in the other chamber, the dried air was passed through a long tube filled with alternate layers of soda-lime, glass wool, and solid sodium hydroxide, to remove the carbon dioxide from the air. Since the chambers were by no means air-tight, and had to be opened to water the plants, etc., doubtless there was some carbon dioxide in the chamber in any case. To avoid this as much as possible, further sodium hydroxide solution was placed within the case. Into each case eight cans of plants were placed, that is, two cans of each of the four species. Both cases received light from a 1000-watt lamp for 14 hours each day. During illumination the temperature in the cases went up to about  $2^{\circ}$  or  $3^{\circ}$  C.

In a warmer room, at about  $20^{\circ}$  C., two similar cases were arranged, one with ordinary air and the other with carbon dioxide-free air. These plants



FIG. 3. Condition of tomato plants from experiments 3 and 4 after 2 days of treatment. On extreme left is the pot of plants which received no carbon dioxide; next to it, the one which received carbon dioxide. The plants receiving no carbon dioxide suffered very severe injury in the cold room, although they did not freeze; the others were uninjured. The two pots on the right are from experiment 4. The plants in the pot on the extreme right received illumination for four periods of 2 hours each at  $0^{\circ}$  C. with the other 16 hours each day at  $22^{\circ}$  C. They were badly injured by the cold. The other plants received an equal illumination, but at  $22^{\circ}$  C., with the remaining 16 hours at  $0^{\circ}$  C. They were uninjured by the cold. In this case, an opportunity for high photosynthesis and low respiration may well explain their ability to survive the cold for 16 hours, whereas the plants with an opportunity for high respiration and low photosynthesis could not survive.

received 14 hours of illumination each day at a temperature of approximately  $24^{\circ}\text{C}$ . Each day, at the end of the illumination period, the plants from both of the glass cases in the warm room were transferred to the cold room, where they remained for 10 hours in the dark at  $0^{\circ}\text{C}$ . They were then transferred back to the cases in the warm room, and the process was repeated. At the end of two days the photograph shown as figure 3 was taken. This shows that the tomato plants receiving light in the warm room, but no carbon dioxide, were very badly injured in the cold room, although they were not frozen. This injury was plainly evident after the first night in the cold room, following the first day without carbon dioxide. The plants receiving carbon dioxide were not injured perceptibly by the exposure in the cold room.

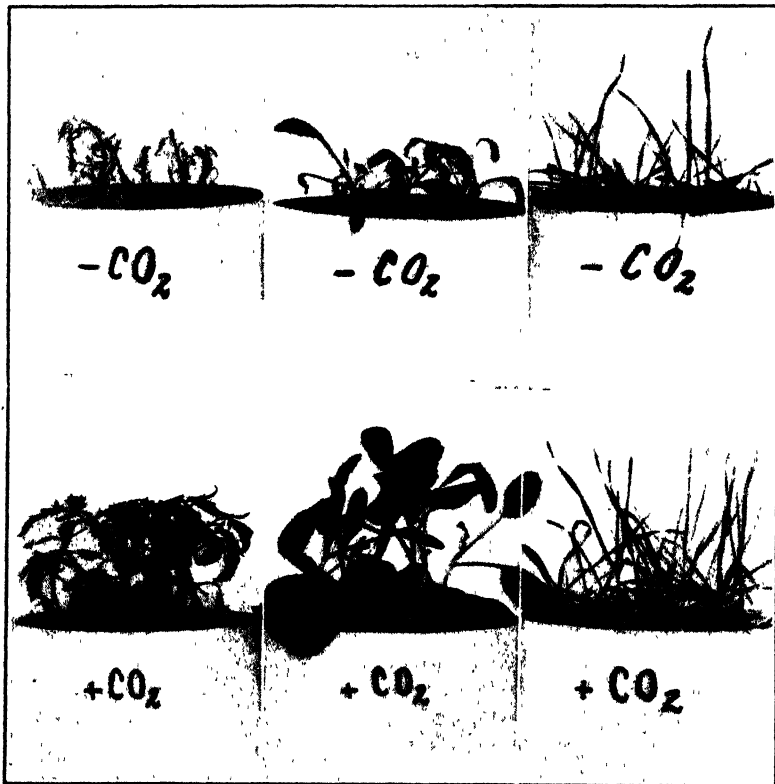


FIG. 4. Condition of the two sets of plants in the room at about  $24^{\circ}\text{C}$ . at the end of two weeks of treatment. Each set received 14 hours of illumination in the warm room, and 10 hours in the dark at  $0^{\circ}\text{C}$ . each day. The upper set received carbon dioxide-free air during the two weeks, and the plants were almost dead. The lower set received ordinary (dehydrated) air and were in good condition.

The other pots in figure 3 received an alternating temperature and light treatment as described in experiment 4. The uninjured plants received their light (four 2-hour periods of illumination) in a warm room, while the badly wilted plants next to them, on the extreme right, received equal illumination, but in the cold room. Again the injury was plainly evident after one day of treatment.

Photographs of the plants with and without carbon dioxide in the warm room, taken at the end of two weeks, are shown in figure 4. Here it is evident that the cabbage and tomato plants receiving light but no carbon dioxide were almost dead, and the wheat was wilted and yellow. The pots of alfalfa plants were omitted from the photograph, since the numbers of plants per pot were not identical. The alfalfa plants showed the starvation effect later than the other plants, but it was evident at the end of two weeks.

At the end of one week of hardening treatment, and at the end of two

TABLE IV

SPECIFIC CONDUCTIVITIES ( $\times 10^6$ ,  $2^\circ \text{C.}$ ) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF SAMPLES OF THE PLANTS IN EXPERIMENT 3. SAMPLES OF WHEAT AND ALFALFA WERE FROZEN FOR 2 HOURS AT  $-8^\circ \text{C.}$ ; THOSE OF CABBAGE AND TOMATO FOR 4 HOURS AT  $-5^\circ \text{C.}$ ; FOUR-TEEN HOURS OF ILLUMINATION DAILY

SPECIES	CARBON DIOXIDE	DAYS OF HARDENING TREATMENT			REMARKS
		0	7	14	
Continuously in cold at 0° C.					
Alfalfa . . . .	Present	297	274	210	Both sets almost dead
Alfalfa ..	Absent	297	314	317	
Wheat . . .	Present	650	367	226	
Wheat	Absent	650	499	345	
Cabbage	Present	804	658	486	
Cabbage .	Absent	804	1027	1445	
Tomato .	Present	1060	1006	1250 }	
Tomato . . . .	Absent	1060	1159	1385 }	
Illuminated at 24° C., and 10 hours in dark at 0° C.					
Alfalfa . . . . .	Present	297	263	296	Decided elongation in both cases
Alfalfa . . . . .	Absent	297	292	320	
Wheat . . .	Present	650	567	621 }	Approximately doubled in size
Wheat . . . .	Absent	650	635	700 }	
Cabbage . . . .	Present	804	976	707	Almost dead
Cabbage . . . .	Absent	804	1093	1709	
Tomato . . . . .	Present	1060	1052	946	Approximately doubled in size
Tomato . . . . .	Absent	1060	1081	1385	

weeks, samples from the four sets of plants were prepared for testing, as previously described. The conductivity determinations are given in table IV.

From the figures in table IV, it is evident that hardening was favored by the presence of carbon dioxide in the air. The different reactions of the four species are of considerable interest. Thus wheat and alfalfa, and to a less extent cabbage, hardened much more thoroughly when kept continuously in the cold. The tomato plants, however, were severely injured by the conditions of hardening before the period was over, with the exception of the plants receiving carbon dioxide and light at the higher temperature. The tomatoes would not survive the occasional exposures to a temperature of 0° C. unless given illumination at a higher temperature for a part of the day. Experiment 4, which follows, brings this point out further. It is also of interest to note that, of the plants in the cold room continuously, but largely without carbon dioxide, the winter wheat alone hardened. Wheat plants of the same set in the same room in the dark showed no hardening whatsoever. In experiment 2 it was shown that when carbon dioxide was completely excluded, wheat hardened almost precisely the same in the light as in the dark.

As the photographs in figure 4 show, the tomato and cabbage plants grown in the warm room, with carbon dioxide, grew extensively in the two weeks' period, and almost doubled their green weight. This was, assuredly, unfavorable for hardening. It may well be also that the long day was a further aggravating tendency. The work of HARVEY (4) would indicate that a temperature of from 5° to 10° C. lower might have caused the cabbage plants to harden much more completely under the conditions of alternating temperatures; in fact, fully as completely as those kept continuously in the cold room. His photographs indicate that there was no perceptible difference in the size of the plants hardened with constant low, and alternating high-low temperatures. This difference in the growth behavior of the plants would readily account for the difference in hardening response in the two experiments.

The winter wheat plants which received a long day at 24° C. elongated their leaf sheaths very notably, whether they received carbon dioxide or not. The plants receiving no carbon dioxide, however, were much more chlorotic.

#### Experiment 4

In this experiment, four species of plants were again used, and of the same kinds and ages. However, the alfalfa was of another variety, and the cabbage and tomatoes were grown in soil. Only the wheat samples were identical with those used in experiment 3. The samples were pre-

pared as before, except that 4-gram duplicate samples of the tomatoes and cabbage were used. Freezing and other details were carried out as described for experiment 3.

In this experiment, an attempt was made to provide conditions during hardening which would combine the effects of alternating temperatures and "cold shocks" with those of increased photosynthesis or respiration. The plants were hardened in five ways: (1) Illuminated for 2 hours at 22° C., then moved into the dark in the cold room at 0° C. for 2 hours, again into the light in the warm room; repeating four times. Thus the plants received 8 hours of interrupted illumination in the warm room, with a total of 16 hours each day in the dark at 0° C. (2) Precisely as the previous case, except reversed; that is, illumination was given only in the cold room, darkness only in the warm room. There was, therefore, 8 hours of interrupted illumination at 0° C. and 16 hours in the dark at 22° C. (3) These plants received the same temperature treatment as (1) but no light; that is, 8 hours, interrupted, in the room at 22° C. and 16 hours at 0° C., all in the dark. (4) Continuous dark, at 0° C. (5) Continuous light at 0° C.

In no case were the plants which were moved kept in absolute darkness; they were covered with heavy cardboard boxes, but could receive a fraction of a foot-candle of illumination when presumably in darkness.

Figure 3 shows the sharp difference in behavior in the tomato plants of treatments 1 and 2, photographed at the end of the second day. From this reaction it would appear that the alternating temperature behavior might well be associated with increased photosynthesis and reduced respiration in treatment 1 as contrasted with slow photosynthesis and high respiration in treatment 2. Figure 5 shows the plants from treatments 1, 2, and 3 at the end of 13 days of hardening treatment. Again the pots of alfalfa plants are omitted. (During the second week, there were two periods of illumination of 4 hours each, rather than four periods of 2 hours each.) When the photographs were taken, the tomato and cabbage plants of treatment 1 had made considerable vegetative elongation; those of condition 3 (alternating temperature in darkness) were almost dead; and the tomatoes in treatment 2 (light given at 0° C.) were also almost dead. In the two remaining treatments, 4 and 5, none of the plants made apparent growth, while the tomato plants gradually died, although those receiving light survived longer than those in darkness. Of the five treatments, but one set of tomato plants survived the experiment, namely, treatment 1. This was precisely the result found in the experiment previously described, in which it was shown that the tomato plants appeared to require an opportunity for photosynthesis at a fairly high temperature if they were to survive occasional exposures to a temperature of 0° C.

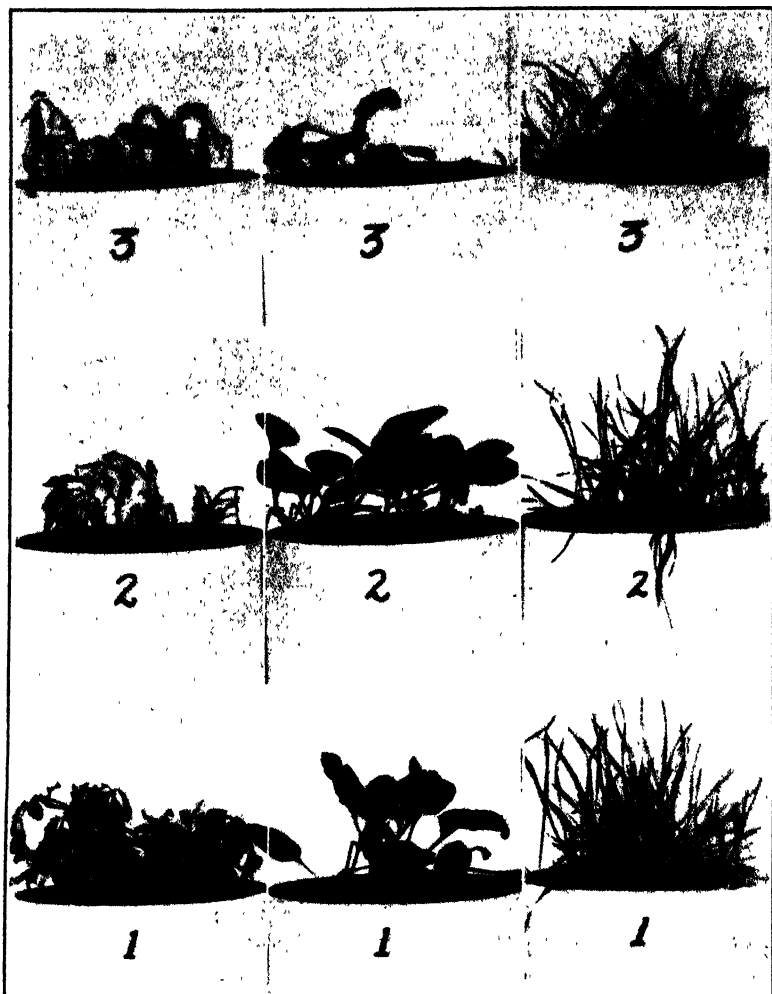


FIG. 5. Condition of the plants after 13 days of treatment. The top row of pots received alternating temperatures in the dark (treatment 3); the middle row received light in a cold room ( $0^{\circ}\text{C}.$ ) in four 2-hour periods, and the remaining 16 hours in the dark at about  $22^{\circ}\text{C}.$ ; the lower row received light in a room at about  $22^{\circ}\text{C}.$  in four 2-hour periods, and the remaining 16 hours in the dark at  $0^{\circ}\text{C}.$  The tomatoes in the bottom row were almost uninjured, whereas the others were severely injured. The cabbage plants in the top row were almost dead. All sets of wheat were adversely affected by the warm temperature treatment, but those of the top row were in a very chlorotic condition and almost dead.

The leaves of the cabbage plants from treatment 3 were so badly wilted and shrunk that it was impossible to get strictly comparable samples, but the conductivity value is included in the table. Comparable samples of the

tomato plants could not be obtained, since, in all except treatment 1, only stumps of the stems remained alive. Table V gives the conductivity values of the samples at the beginning of the trial, and after two weeks of hardening treatment.

TABLE V

SPECIFIC CONDUCTIVITIES ( $\times 10^4$ ,  $2^\circ\text{C}.$ ) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS FROM FROZEN PLANTS IN EXPERIMENT 4; FREEZING TREATMENT AS IN EXPERIMENT 3

PLANT	TREATMENT	DAYS OF HARDENING TREATMENT	
		0	14
Alfalfa	1. Warm light-cold dark	388	265
	2. Cold light-warm dark	388	263
	3. Continuous dark, warm-cold	388	321
	4. Continuous dark and cold	388	289
	5. Continuous light and cold	388	265
Wheat	1. Warm light-cold dark	650	500
	2. Cold light-warm dark	650	633
	3. Continuous dark, warm-cold	650	616
	4. Continuous dark and cold	650	659
	5. Continuous light and cold	650	234
Cabbage	1. Warm light-cold dark	1085	702
	2. Cold light-warm dark	1085	751
	3. Continuous dark, warm-cold	1085	1750
	4. Continuous dark and cold	1085	1047
	5. Continuous light and cold	1085	463
Tomato	All very severely injured in hardening process except (1), warm light-cold dark, which survived very well. No comparable samples obtainable		

Table V shows that hardening was more complete in treatment 1 than in treatment 2. In the case of the alfalfa, the roots were buried in the soil, of course, and did not actually receive the sharply alternating temperatures that the other parts tested did. The wheat and cabbage gave evidence of more complete hardening in 1 and in 2 by their conductivity values; the tomatoes by their survival at the temperatures used (figs. 3, 5). There seems to be no evidence that alternating temperatures as such (treatment 3) were beneficial in hardening these plants. In fact, on the average this would seem to have been the poorest treatment of the five used in this experiment. This seems not unreasonable, since it gave a considerable opportunity for respiration and no opportunity for photosynthesis, except during the intervals when the plants were being moved, or in the slight illumination under the covers. By all means the most efficient hardening

treatment (with the exception of the tomatoes) was the continuous light with continuous cold. Tomatoes, however, did not seem to be able to endure this temperature without severe injury, unless receiving illumination at a higher temperature for part of the day. It seems probable that photosynthesis, with the tomato, is almost negligible at the temperature near  $0^{\circ}\text{C}$ .

### Discussion and conclusions

The results of these experiments all point, in the opinion of the writer, to similar conclusions. The general proposition may be stated that hardening of plants is favored by conditions which tend toward the accumulation or conservation of carbohydrates and other reserve foods; that is, which further photosynthesis and lessen respiration and extension of vegetative parts. Hardening proceeded markedly in the dark in alfalfa or winter wheat plants that had an abundant storage of organic food, if the temperature was near  $0^{\circ}\text{C}$ . The more succulent wheat plants, grown in the greenhouse, gave no indication of hardening in the dark at  $0^{\circ}\text{C}$ ., but hardened rather completely when illuminated. Alternating temperatures were not shown to be particularly favorable to hardening in either light or dark. A short period, with light, at a higher temperature was not especially deterrent in the hardening process, except in the case of winter wheat. This cannot be said of a longer day at the higher temperature, for in this case, with both alfalfa and wheat, and perhaps with cabbage and tomato, elongation of foliar parts was especially evident. The species are by no means identical, however, in their reactions. Winter wheat was much more adversely affected in its hardening behavior than alfalfa, cabbage, or tomato by a period of short duration at a higher temperature. In fact, tomato plants could not survive occasional exposures to a temperature of  $0^{\circ}\text{C}$ . unless they had opportunity for photosynthesis at higher temperatures.

Exposure to a long day in the hardening room at  $0^{\circ}\text{C}$ . gave no indication of the usual response in wheat and alfalfa of decided elongation of parts. On the contrary, the plants hardened more fully with such long-day illumination treatment than with a short day. Winter wheat plants which had received a short day in the greenhouse ( $60^{\circ}\text{F}$ .) hardened more rapidly and more fully in the cold room than similar long-day (greenhouse) plants under either a long- or a short-day hardening treatment. The short-day plants were smaller, greener, and somewhat higher in dry matter.

Further evidence of the relation of organic foods to hardening is given by experiments 3 and 4. Winter wheat, alfalfa, tomato, and cabbage plants were hardened in several ways which would favor photosynthesis

and decrease respiration, or the reverse. Removal of carbon dioxide from the air given the plants in all cases prevented or greatly depressed the degree of hardening. Plants receiving their light in a warm room and their dark period in a cold room were in all cases hardier than those receiving the opposite treatment. The first would seem to favor photosynthesis and depress respiration, while the second would tend to use the reserve foods of the plant without replenishment. Alternating temperatures, as such, without illumination did not appear to be helpful in the hardening of the plants. When marked top growth occurred, even under conditions favoring photosynthesis and depression of respiration, hardening was lessened.

Although the various species used differed considerably in their behavior, the general suggestion seems to hold that the development and maintenance of a high available carbohydrate supply, with much retarded vegetative growth, is essential before the cold-temperature reaction of hardening of plants will occur in an efficient manner.

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# INFLUENCE OF VARIOUS NITROGENOUS COMPOUNDS AND MANNITOL ON NODULE FORMATION BY CLOVER<sup>1</sup>

E. W. HOPKINS AND E. B. FRED

(WITH TWO FIGURES)

## Introduction

In a previous paper (9), the influence of nitrate upon the number and distribution of nodules on red clover plants was considered. It seemed desirable to continue this work, using a number of organic nitrogenous compounds.

The wide occurrence of organic nitrogenous compounds in soil makes it imperative that these compounds as well as nitrates be considered in their effect upon nodule formation. Investigations on this problem have been limited. FRANK (4) found that peas fixed more nitrogen when urea was added to sand cultures, and that lupines behaved in an opposite manner. FLAMAND (2) determined that the following concentrations of urea and oxamide inhibited nodule formation in Sach's solution: *Pisum sativum*, urea less than 1/20,000, oxamide 1/20,000; *Vicia narbonensis*, urea and oxamide 1/20,000; *Faba equina*, urea 1/20,000, and oxamide 1/10,000. Similarly, the carbonaceous materials of soil may play a rôle in nodulation. This problem has also received little attention. RITTER (14) reported that in soil, sucrose had no effect on the formation of nodules by lupines. PRUCHA (13), however, found that more than 2 gm. of sucrose added to 300 gm. of soil adversely affected nodulation of peas, while 0.2 to 2 gm. of sucrose apparently had no influence. According to WILSON (18), maltose, fructose, lactose, sucrose, and glycerol stimulated nodule formation on soy beans, while glucose was inactive. Calcium saccharate was found to possess a particularly stimulating effect.

## Experimentation

In the following study, the attempt was made to produce a plant environment such that the nodule bacteria would not find invasion of the plant roots necessary in order to obtain carbohydrate and fixed nitrogen. Although such environments modified nodule formation, in no case was actual inhibition of nodules observed.

The nitrogen compounds added to the plant cultures were  $\text{KNO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ , urea, asparagin, clover-seed extract, and yeast extract. In a second series, mannitol was added in addition to the nitrogen compounds.

<sup>1</sup> Herman Frasch Foundation in Agricultural Chemistry, Paper no. 29. Contribution from the Departments of Agricultural Bacteriology and Agricultural Chemistry, University of Wisconsin.

**METHODS.**—The methods and details of planting are given in the previous paper (9). In the tests where mannitol was added, the concentration used was 0.5 per cent. To avoid decomposition of the nitrogenous compounds during sterilization, these were added with aseptic technique to the sterile agar shortly before hardening occurred. Sterilization of the solutions of  $\text{KNO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ , urea, and asparagin before addition to the agar was effected by filtration through a Berkefeld filter. The asparagin solution was neutralized with  $\text{NaOH}$  to the phenolphthalein end-point. The yeast and clover-seed extracts could not be filtered, and so were sterilized in the autoclave. Solutions of the nitrogen sources were added in such amounts to the bottles that series of 2, 5, 10, and 20 mg. N per bottle were obtained.

The yeast extract was made up as given by FRED and WAKSMAN (5). In the series with no mannitol added, the clover seed extract was prepared by soaking the seed and then steaming. For the mannitol series, the extract was obtained from macerated clover seedlings. The extracts were filtered through paper pulp. The nitrogen present in these extracts was determined by the Kjeldahl-Gunning method.

**PLANT GROWTH.**—The large number of bottles required for the experiment made it necessary to extend the planting over a period of several weeks. Ten bottles of each treatment were included; thus a total of over 500 bottles was required. At the time of harvest, the difference in age of the cultures was about one month. This difference in age, however, would not appear to be an important consideration, since at the time of harvest all plants had attained the maximum development possible under the conditions. The cultures ranged in age from 90 to 120 days. The growth period was during November, December, January, and to the middle of February. During this period, plant growth is very slow owing to deficient light. The plants were exposed to artificial illumination, and changed about to maintain conditions as constant as possible throughout the growth period.

At the end of the experiment, the plants were pulled from the agar. There was very little loss of root material, because the agar was soft enough to allow passage of the roots without breaking them. The plants were examined for their general appearance, and nodule counts were made. The size, shape, and distribution of the nodules were noted. Plants from three bottles were combined, the total nitrogen determined by the Kjeldahl-Gunning method, and the results calculated for ten plants.

The effects of the various compounds upon the growth of the plants will be considered under the discussion of each nitrogen source, and the effect on nodule formation in the general discussion.

**CONTROLS.**—The growth of the controls containing no mannitol was very good. At all times the plants possessed a deep green color, and at the time

of harvest, filled the bottles. In the controls containing mannitol, growth was somewhat slower, but the general condition of the plants and their color was good; table I gives the data for the two sets of controls.

TABLE I  
EFFECT OF MANNITOL ON NODULE FORMATION

TREATMENT	TOTAL N IN 10 PLANTS (1 BOTTLE)	NUMBER OF SAMPLES	NODULE MEAN, 10 PLANTS	DISTRIBUTION AND SIZE OF NODULES
Uninoculated control .....	<i>mg.</i> 2.3	...	.....	.....
Control 1: no mannitol	9.7	9	$138 \pm 11$	Nodules principally on tap-roots, many near crown, mostly of the round type; roots normal
Control 2: 0.5 per cent. mannitol ..	9.4	9	$95 \pm 6$	Nodules principally on tap-roots, many near crown, mostly of the long type and very large; roots slightly stunted in a few plants

INORGANIC NITROGEN COMPOUND SERIES.—In these series,  $\text{KNO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  were present in varying amounts. The plants grew very well in the nitrate series without mannitol, and in color and size were even better than the controls. In the mannitol series, with increasing nitrate, the plants did not grow well. In this series, as in all others containing mannitol, the organisms grew over the surface of the agar and along the plant roots, increasing vigor of growth of bacteria being evidenced in the higher concentrations of the nitrogen source. This growth apparently was unfavorable for the plants, because as the amount of growth increased, the roots became more stunted, and frequently were gnarled and very irregular, or failed to put out secondary roots. In some cases, however, the plants were able to attain good growth in spite of this condition. None of the concentrations of nitrates used was found to prevent bacterial growth, so it is probable that the organisms were not injured by this salt, as HILTNER

TABLE II  
EFFECT OF INORGANIC NITROGEN COMPOUNDS ON NODULE FORMATION

NITROGEN ADDED PER BOTTLE	TOTAL N IN 10 PLANTS (1 BOTTLE)	NO. OF SAMPLES	NODULE MEAN, 10 PLANTS	CRITICAL RATIO, STANDARD DEVIATIONS	DISTRIBUTION AND SIZE OF NODULES
<i>mg./250 cc.</i>	<i>mg.</i>				
Uninoculated control	2.3				
Series 1: $\text{KNO}_3$ , no mannitol					
2	10.4	9	$152 \pm 8$	+1.03	Nodules present principally on secondary roots, largely of round type, some distributed over root system, decreasing in size with increasing nitrate; roots normal
5	9.0	10	$161 \pm 11$	+1.47	
10	11.1	10	$130 \pm 13$	-0.41	
20	11.4	11	$110 \pm 12$	-1.72	
Series 2: $\text{KNO}_3$ , 0.5 per cent. mannitol					
2	8.5	9	$90 \pm 11$	-0.40	Nodules principally on tap-root, many near crown, large, mostly of long type, decreasing in size with increasing nitrate; roots stunted with increasing nitrate
5	9.3	10	$80 \pm 5$	-1.92	
10	6.4	9	$55 \pm 4$	-5.32	
20	5.0				
Series 3: $(\text{NH}_4)_2\text{SO}_4$ , no mannitol					
2	6.2	10	$64 \pm 5$	-6.15	Nodules principally on secondary roots, mostly of round type but some long, decreasing in size with increasing ammonia; roots slightly stunted
5	6.8	10	$70 \pm 8$	-5.00	
Series 4: $(\text{NH}_4)_2\text{SO}_4$ , 0.5 per cent. man- nitol					
2	3.9	10	$50 \pm 6$	-5.30	Nodules principally on tap-root, in 2 mg., many near crown, largely of long type, many large round type increasing with increasing ammonia; roots stunted with increasing ammonia
5	5.6	7	$43 \pm 5$	-6.65	
10	5.2				
20	3.1				

(8) has assumed. The other nitrogen sources appeared to be equally as good or better than nitrate in producing growth of the organism. Thus no toxic effect on the organism was observed with any nitrogen compound used.

Bacterial growth had covered the roots of the plants in the 20-mg. set, with the result that the plants were badly stunted. The set was discarded.

In the  $(\text{NH}_4)_2\text{SO}_4$  series, without mannitol, all the plants in the 20 mg. of  $\text{NH}_3\text{-N}$  set and most of those in the 10-mg. set died. When mannitol was present, none of the plants was killed; but when harvested, they were so small that nodule counts were not made. In the lower concentrations of ammonia, in both the mannitol and non-mannitol series, the plants attained a fair growth but were of a pale green color. Table II gives the data of the  $\text{KNO}_3$  and  $(\text{NH}_4)_2\text{SO}_4$  series.

ORGANIC NITROGEN COMPOUND SERIES.—In these series, urea and asparagin (as the sodium salt) were added to the agar. In both urea series, with and without mannitol, the plants attained a good size, and were dark green in color. The higher concentrations of urea, however, were toxic to the plants, and in the series without mannitol the plants died in the 20 mg. of urea-N set. The plants in the 10-mg. set developed rather slowly at first, but after slow growth at the start, were able to outgrow the other series. In the urea series with mannitol, all sets above 5 mg. of urea-N grew very poorly, and at the end of the experiment the plants were only a few cm. high.

In the asparagin series excellent plant growth was obtained in most of the sets. The plants in the 20 mg. of asparagin-N died, but the color of the plants in all sets was even darker than that of the controls. This series is especially interesting because some workers have considered amides to be the first compounds formed in the fixation of nitrogen by leguminous plants. FRANK (3) expressed the view that nitrogen was fixed as amides in the leaves of the Leguminosae. STOKLASA (15) believed that amides were the first compounds formed in nitrogen fixation. It is somewhat surprising that the concentration of 20 mg. of asparagin-N would be high enough to kill the plants, especially since the nitrogen present was only about one-quarter to one-half more than ten plants would require for their growth under the conditions used. When mannitol was present with asparagin, poor plant growth took place in the higher concentrations.

The excellent growth obtained in series 7 is no doubt due to the utilization of asparagin by the plants. VIRTANEN and VON HAUSEN (17) have recently shown that aspartic acid and the amino acids in a casein digest are readily assimilated by red clover. It is very probable, they believe, that free nitrogen is fixed in the nodules as amino acids.

The data for the urea and asparagin series are given in table III.

TABLE III  
EFFECT OF ASPARAGIN AND UREA ON NODULE FORMATION

NITROGEN ADDED PER BOTTLE	TOTAL N IN 10 PLANTS (1 BOTTLE)	NO. OF SAMPLES	NODULE MEAN, 10 PLANTS	CRITICAL RATIO, STANDARD DEVIATIONS	DISTRIBUTION AND SIZE OF NODULES
<i>mg./250 cc.</i>	<i>mg.</i>				
Uninoculated control.	2.3				
Series 5: urea, no mannitol					
2	9.3	10	111 ± 10	-1.81	Nodules principally on secondary roots, some distributed over root system, largely of round type, decreasing in size with increasing urea; roots normal
5	9.0	10	110 ± 9	-1.95	
10	9.5	11	51 ± 7	-6.70	
Series 6: urea, 0.5 per cent. mannitol					
2	7.9	10	58 ± 5	-4.73	Nodules principally on tap-root, mostly round type, though many long type, decreasing in size with increasing urea; roots stunted with increasing urea
5	5.5	9	62 ± 7	-3.67	
Series 7: asparagin, no mannitol					
2	9.8	11	106 ± 8	-2.35	Nodules principally on secondary roots, largely of round type, in- creasing distribution along root system with increasing aspara- gin; roots normal
5	10.8	10	100 ± 9	-2.68	
10	14.2	10	111 ± 15	-1.45	
Series 8: asparagin, 0.5 per cent. man- nitol					
2	6.9	10	72 ± 5	-3.58	Nodules principally on tap-root, many near crown, principally of long type, changing to round type with increasing asparagin; roots stunted with increasing asparagin
5	7.2	10	128 ± 20	+1.58	
10	6.4	10	53 ± 9	-3.89	
20	6.7				

**PLANT EXTRACT SERIES.**—To these series, clover-seed extract and yeast extract were added to the agar. With both of these nitrogen sources, plants in the non-mannitol series attained a moderate growth, but were of a rather pale color. In the mannitol series, all plants grew very well and possessed a dark green color. In appearance, the plants were better than those of any other series. Table IV gives the data on the clover-seed and yeast extract series.

### Discussion

The data in these experiments may be discussed according to the nitrogen compounds used. In another part of this paper, the results will be considered as a whole.

For the mean of the number of nodules in each set, the standard deviation was computed, and the effectiveness of the treatment determined by the method of the critical ratio. This was calculated by comparing each set with its control. A critical ratio of 2.5 standard deviations was taken as the limit of significance.

**CONTROLS.**—The data are given in table I. The amount of nitrogen contained in the plants in these sets will be used as the comparison for that of the sets receiving nitrogen compounds. Mannitol decreases the number of nodules which form, but these nodules are larger than those of plants without mannitol. Other workers have commented on the increased size of nodules produced by carbohydrates. HARRISON and BARLOW (7), growing pea and vetch in 0.4 per cent. maltose-wood ash agar, observed that very large nodules were produced. LEONARD (11) found that when glucose was added to sand cultures of soy beans in bottles, the roots were injured, although the nodules which formed were twice the normal size. The nodules in the controls are located principally on the tap-root, with many close to the crown. In the controls without mannitol, the round nodules predominated, while in those with mannitol present, the long type were more numerous. The statistical constant was not calculated for the controls.

**INORGANIC NITROGEN COMPOUNDS SERIES.**—Comparing the nitrogen content of series 1 and 2 (table II), it is apparent that the plants with mannitol assimilated less nitrogen than those without. The heavy growth of bacteria which occurred on the surface of the agar and along the plant roots of the mannitol-nitrate set apparently exerted a toxic effect upon the plants. The number of nodules is decreased significantly only in the 10-mg. set in series 2. With all of the nitrogen present at the start, it was found in a previous experiment (9) that the number of nodules was irregularly affected. The distribution of the nodules in series 1 is affected as noted previously (9); that is, that even a small amount of nitrate causes the nodules to form principally on the secondary roots, such nodules being of the small round

TABLE IV  
EFFECT OF CLOVER-SEED AND YEAST EXTRACTS ON NODULE FORMATION

NITROGEN ADDED PER BOTTLE	TOTAL N IN 10 PLANTS (1 BOTTLE)	No. OF SAMPLES	NODULE MEAN, 10 PLANTS	CRITICAL RATIO, STANDARD DEVIATIONS	DISTRIBUTION AND SIZE OF NODULES
<i>mg./250 cc.</i>	<i>mg.</i>				
Uninoculated control.	2.3				
Series 9: clover-seed extract, no mannitol					
2	6.0	10	84 ± 8	-4.00	Nodules principally on tap-root, changing to secondary root with increasing extract, mostly of long type, changing to round type, some very large; roots stunted in increasing extract
5	7.3	10	64 ± 5	-6.12	
10	8.3	10	60 ± 7	-6.00	
20	7.3	5	30 ± 5	-9.00	
Series 10: clover-seed extract, 0.5 per cent. mannitol					
2	5.6	9	61 ± 6	-4.01	Nodules principally on tap-root, many near crown, predominantly of long type, many very large; roots stunted with increasing ex- tract
5	11.7	9	71 ± 6	-2.83	
10	10.7	9	65 ± 4	-4.16	
20	10.4	10	43 ± 4	-7.21	
Series 11: yeast ex- tract, no mannitol					
2	7.9	10	87 ± 7	-3.53	Nodules principally on tap-root, changing to secondary root with increasing extract, mostly of long type, changing to round type, very small with increasing ex- tract; roots very slightly stunted
5	8.3	10	108 ± 9	-2.04	
10	11.7	8	74 ± 6	-5.12	
20	13.0	4	54 ± 12	-5.16	
Series 12: yeast ex- tract, 0.5 per cent. mannitol					
2	8.2	10	50 ± 5	-5.76	Nodules principally on tap-root, predominantly of long type, many large; roots stunted with increasing extract
5	8.4	8	44 ± 3	-7.60	
10	14.3	10	82 ± 7	-1.41	
20	13.0	9	43 ± 5	-6.65	

type. With increasing nitrate, the size of the nodules decreases. When mannitol is present, however (series 2), the distribution of the nodules is not affected by the nitrate, although the size of the nodules decreases in the higher nitrate sets. The placement of the nodules is essentially normal,—mostly on the tap-root and near the crown. Mannitol increases the size of the nodules.

In series 3 and 4 (table II) the effect of  $(\text{NH}_4)_2\text{SO}_4$  on nodule formation is shown. The plants did not grow as well as those in series 1 and 2, and did not assimilate as much nitrogen. The number of nodules was significantly decreased by all concentrations of  $(\text{NH}_4)_2\text{SO}_4$ . The effect upon position of the nodules was the same as that produced by nitrates, scattered nodules in series 3, and nearly normal nodules in series 4 with mannitol present.

ORGANIC NITROGEN COMPOUNDS SERIES.—In both the urea and asparagin series, the plants without mannitol (series 5 and 7, table III) assimilated more nitrogen than those to which mannitol was added. The amount of nitrogen in ten plants increased as more of the nitrogen compound was available. Asparagin seemed to be an especially good source of nitrogen.

The number of nodules was irregularly affected by the nitrogen treatment. As judged by the critical ratio, the number of nodules was decreased in series 5 by 10 mg. of urea-N, in series 6 by 2 and 5 mg. of urea-N, in series 7 by 2 and 5 mg. of asparagin-N, but not by 10 mg.; and in series 8 by 2 and 10 mg. of asparagin-N, but not by 5 mg. Asparagin seems to be less consistent than any of the nitrogen sources used in the effect on nodule formation.

The distribution of the nodules on the roots was very similar to that in the  $\text{KNO}_3$  series (series 1 and 2, table II). The nitrogen sources produced scattered round nodules, even in the lowest concentrations, with decreasing size of the nodules when more of the nitrogen source was present. When both nitrogen compound and mannitol were present, as seen in the  $\text{KNO}_3$  series, the nodules were placed largely on the tap-roots and attained a good size. However, the size of the nodules decreased as the concentration of the nitrogen compound increased.

PLANT EXTRACT SERIES.—The plants in series 9–12 exhibited good growth in both the series with and those without mannitol. When mannitol was present in addition to the nitrogen sources, the highest nitrogen plants were produced.

The number of nodules showed certain irregularities, but was generally depressed by all concentrations of the plant extracts. In all clover-seed extract sets, 2, 5, 10, and 20 mg. N with and without mannitol, the number of nodules was decreased. In the yeast extract series (series 11 and 12), the 5-mg. yeast extract-N set without mannitol, and the 10-mg. set with

mannitol showed no significant effect on the number of nodules. All other concentrations of yeast extract effectively decreased nodule formation.

In the distribution of nodules, it will be noted that the plant extracts produced less deviation from normal placement than did the other nitrogen sources. In the 2-mg. sets, without mannitol, the distribution of the nodules was essentially normal; but as the concentration of the plant extracts increased, the distribution of nodules was affected the same as in the case of

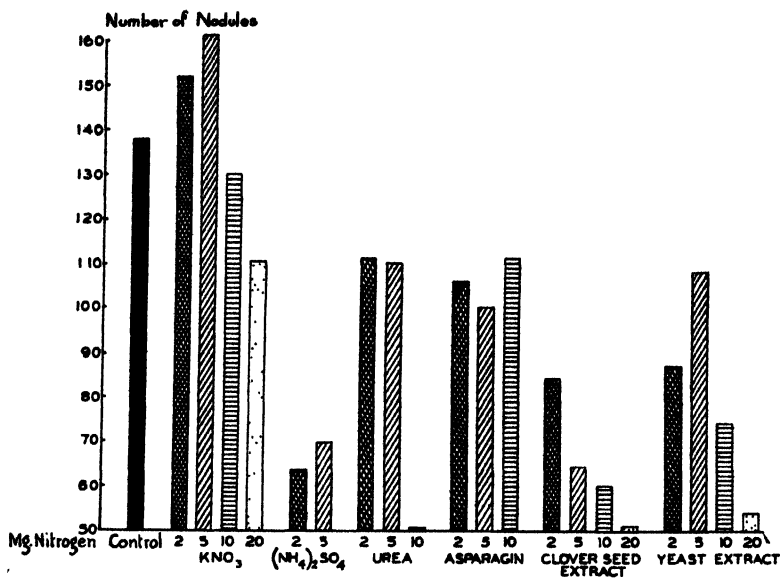


FIG. 1. Effect of various nitrogen compounds on nodule formation.

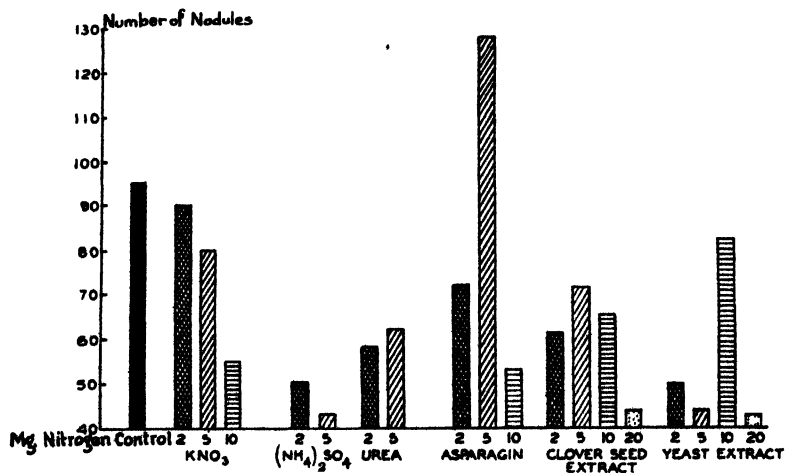


FIG. 2. Effect of various nitrogen compounds and mannitol on nodule formation.

the other nitrogen sources. Nodules then appeared principally on the secondary roots, and were small and round. When mannitol was present in addition to the plant extracts, no change from the normal placement of nodules occurred, but increasing concentrations of the plant extract resulted in smaller nodules.

Figure 1 shows the comparison of the nitrogen sources in the absence of mannitol, and figure 2, the series with mannitol and a nitrogen source.

From the foregoing data, certain generalizations may be made. When plants were grown in agar containing any one of the six nitrogenous compounds used, nodules on such plants were affected in two ways. All of the nitrogen sources were found, first, to decrease the size of the nodules formed, and second, to affect the distribution of the nodules on the roots. The number of nodules was influenced irregularly. Mannitol was found to have three effects: (1) to decrease the number of nodules, (2) to increase the size of the nodules which formed, and (3) to alter the position of the nodules on the roots.

The normal position of the nodules was near the crown of the root, with most of the nodules on the tap-root or very close to it on the secondary roots. In the normal plants there were a number of the long type of nodules, which are simply large nodules, while the largest number were of the round type, or small nodules. When the nitrogen sources were added, this distribution was changed as was noted for nitrate in a previous publication (9). Even in the lowest concentration (2 mg. of N) of  $\text{KNO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ , urea, and asparagin, the nodules were found, not principally on the tap-root but almost exclusively on the secondary roots. Yeast and clover-seed extracts did not noticeably change the distribution of nodules when 2 mg. of extract N were present, but in all higher concentrations the nodules formed on the secondary roots and were of decreased size. It is noteworthy that clover-seed extract influenced the size of nodules less than did any other nitrogen source.

Considering the series containing both mannitol and a nitrogen source, it will be observed that with mannitol present, the distribution of the nodules was more like that in control plants. While the nodules were fewer, many attained a very large size. The nodules were situated mostly on the tap-root, near the crown. It will be noted further that increasing amounts of the nitrogen source still decrease the size of nodules when mannitol is present, but do not change their position on the roots.

HILTNER (8) proposed that the plant was rendered immune to root invasion when nitrate was available. The view that nitrates are toxic to the nodule bacteria and thus influence nodule formation by injuring the inciting agent has been proposed. HILTNER believed that the nodule bacteria were injured in the soil, while STROWD (16) suggests that the high

concentration of nitrate attained in plant sap is sufficient to injure the invading nodule bacteria. LAURENT (10) expressed the view that nitrates and some constituent of the plant sap form a combination which is toxic to the nodule organisms. Experiments are offered to support this view.

GIÖBEL (6) has offered the ingenious theory that, since it appears probable that nitrogen is fixed in the nodules as an organic compound, such a compound would experience greater difficulty in diffusing through cell walls than would a rapidly transferable ion like  $\text{NO}_3$ . This condition would result in an accumulation in the nodule of the products of fixation, with the result that fixation of nitrogen would be impaired and development of nodules stopped.

MAZÉ (12) has considered the question from an entirely different point of view. His conception relates root invasion to the carbohydrates present in the plant sap. When the plant is receiving nitrate the carbohydrate may be assimilated at once, so that the sap of plants with adequate nitrogen supply would be low in carbohydrate. On the other hand, plants deficient in nitrogen would contain an excess of carbohydrate circulating in the sap. In the latter case the organic matter, especially carbohydrates, secreted by the plant roots would be higher and attract more nodule bacteria. Invasion of the roots would thus be facilitated in high-carbohydrate plants.

Returning to our own data, how are we to interpret the results obtained? The effect of the nitrogen sources on the distribution of nodules seems to be due to prevention of development of nodules at the points of early invasion of the plant roots. The nodules which did form are typical of those produced by late root penetration.

When mannitol and a nitrogen source are added to the plant cultures, the effect of the latter upon the distribution of the nodules is offset. It thus appears that nitrogen compounds *prevent* the formation of nodules at the points of early invasion, while mannitol with the nitrogen compounds allows nodules to *develop* at the points of early invasion. This observation suggests that the early invading bacteria do not form nodules, because the carbohydrate supply of the plant is insufficient to allow the organisms to develop to the extent necessary to incite nodule formation. MAZÉ states that plants receiving fixed nitrogen will be low in carbohydrate. STROWD found that as the nitrogen available to soy beans was increased, the sugar content of the sap fell off sharply. EATON (1) found that as the total sugars and readily hydrolyzable material (hemicellulose, starch, and dextrin) increased in soy beans receiving increasing length of day, the weight of nodules also increased.

The following hypothesis is advanced as an explanation of the results noted. Nitrogen compounds, at least any of the six used, are taken up by the plants, and thus allow the carbohydrates synthesized to be assimilated

at once. If at this time nodule bacteria penetrate the root hairs, they are unable to produce nodules because of the low concentration of sugars in the plant sap. Thus nodules are not formed by the early invaders. As the plant continues to grow it uses up some of the nitrogen source, and no longer has a supply of nitrogen adequate to support its growth at the incipient rate. The carbohydrates increase in the plant sap; and as this continues, a concentration is reached which enables the nodule bacteria penetrating the root hairs at that time to proliferate and incite nodule formation.

### Summary

1. A nutrient solution containing agar was used as a substrate for the culture of red clover plants in bottles. Various nitrogen sources were added to the agar in four concentrations, 2, 5, 10, and 20 mg. of nitrogen per bottle. The nitrogenous substances used were:  $\text{KNO}_3$ ,  $(\text{NH}_4)_2\text{SO}_4$ , urea, asparagin, clover-seed extract, and yeast extract. In a second experiment, the same nitrogen compounds were used but 0.5 per cent. mannitol was also added. At the time of harvest, the general appearance of the plant and the number, size, and distribution of nodules were noted.

2. The effect of the nitrogen compounds upon the number of nodules showed considerable variability, and the results were not entirely consistent with increasing concentration of the nitrogen compounds.

3. It was found that all of the nitrogen sources used decreased the size of the nodules formed, and that this effect became more in evidence as the concentration of the nitrogen compound was increased. The nitrogen compounds were also found to affect the distribution of the nodules on the roots. In the untreated control, the nodules were situated for the most part on or near the tap-root; a few large or long nodules were present, with the greater number of them of a fair size but round in shape. As compared with this condition, in the presence of the nitrogen compounds the nodules were principally on the secondary roots, and were almost exclusively round and small. This latter condition was attributed to later invasion of the roots by the organism, owing to the presence of the nitrogen compounds. In the series containing both nitrogen source and mannitol, it was found that the nitrogen source no longer affected the distribution of nodules. In these series the nodules were principally on the tap-roots. Increasing concentrations of the nitrogen compounds did, however, decrease the size of the nodules formed.

The senior author wishes to thank Professor W. H. PETERSON, Mr. P. W. WILSON, and Mr. P. WENCK for technical assistance rendered and for helpful suggestions made during the course of the experiments.

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# MANGANESE AND THE GROWTH OF LEMNA<sup>1</sup>

NORMAN ASHWELL CLARK

(WITH TWO FIGURES)

Since this paper was submitted to the editor, the paper by McHARGUE and CALFEE (PLANT PHYSIOL. 7: 697-703, 1932) has appeared. As their conclusions are in substantial agreement with the findings reported in this paper in regard to the essential nature of manganese for the growth of *Lemna*, the original manuscript was returned to me and shortened. It should be noted that in these experiments the *Lemna* were grown free from microorganisms, and that therefore the amount of manganese required for maintenance would be expected to be much lower than with the technique of McHARGUE and CALFEE, as was the case.

It has been previously reported (CLARK and FLY 4) that it was not necessary to add manganese to solutions of purified salts in order to secure good reproduction of *Lemna major*, and that the addition of manganese to an inorganic nutrient medium in which *Lemna* grew well produced some effect upon the vegetation but little upon the rate of reproduction until toxic concentrations were reached. The possibility that extremely small amounts of manganese might be essential to the plants and be present in sufficient quantity even in salts recrystallized several times, together with the failure of many workers to obtain growth in green plants without manganese, particularly of HOPKINS (6) with *Lemna minor*, caused the problem to be investigated further.

*Lemna major* (*Spirodela polyrhiza*) has been grown in these laboratories with inorganic salt solutions for several years. The nutrient solution used as standard consists of magnesium sulphate (24 mg. Mg per liter), potassium nitrate (312.8 mg. K), primary calcium phosphate (16.1 mg. Ca), and ferric chloride (0.6 mg. Fe), adjusted to pH 4.7-4.8 with KOH (3). The salts are recrystallized, usually three times. The water is three times distilled, the final distillation being in pyrex glass, and the solution in preparation and use is handled altogether in this glassware. The substitution of quartz Erlenmeyer flasks for pyrex made no change in the rate of growth of the plants. This medium has been used for the growth of *Lemna minor* by ASHBY (1) at the University of London. *Lemna* has been found valuable for physiological experiments by BOTTOMLEY and MOCKERIDGE in England, OLSEN in Denmark, and by SAEGER, DEUBER, WOLFE, and others in this country.

<sup>1</sup> Contribution from the Chemistry Department, Iowa State College.

In order to test for the presence of manganese, oxidation by periodate was used in phosphoric acid solution (7). It was usually possible to detect as low as 0.001 mg. Mn in 50 cc., and additions of 0.001 mg. caused an alteration in the color. The standard inorganic medium for *Lemna* gave no trace of manganese, indicating less than 0.001 mg. in 50 cc., or a smaller amount than 1 part in 50 million. When a quantity of the salts sufficient to make 20 liters of the standard solution was put into 100 cc. (with the precipitate removed and possible loss checked by adding Mn), the solution contained 0.006 mg. Mn in the 100 cc. The amount of manganese in the standard medium was therefore of the order of 1 in 3000 million. By further purification, solutions were obtained which contained  $5 \times 10^{-5}$  mg. of Mn per liter when the iron was supplied as chloride and  $8 \times 10^{-5}$  with iron as citrate. Experiments were made to determine whether the plants would reproduce at these concentrations, or where the minimum occurred under the conditions of growth.

The material, free from microorganisms (5), was grown in 100 cc. of the sterilized medium in Erlenmeyer flasks. Artificial light was supplied for 14.5 hours daily, at approximately 400 foot-candles at the surface of the plants. Temperature was kept at 25°. The plants were transferred twice a week to fresh solutions except where otherwise stated, and their number was reduced as the flasks filled, usually about once a week. The fronds were counted and the rate of reproduction, K, determined for the equation  $\log_{10} N - \log_{10} N_0 = K(t - t_0)$  (2), where N is the number of fronds and t the days of growth. Cultures were run in duplicate or triplicate, but as these checked, curves are shown for only one flask at each concentration. The pH for the ferric chloride solutions was adjusted to 4.7-4.8, and the ferric citrate solutions to 5.7-5.9.

TABLE I  
REPRODUCTION RATE AND MN CONCENTRATION

SOLUTION NO.	IRON SUPPLIED AS FERRIC CHLORIDE		
	MN PER LITER	APPROXIMATE CONCENTRATION	K × 100
	mg.	p.p.m	
1 . . . . .	$2500 \times 10^{-5}$	1:40 million	9.1
2 . . . . .	$505 \times 10^{-5}$	1:200 "	9.1
3 . . . . .	$255 \times 10^{-5}$	1:400 "	9.1
4 . . . . .	$96 \times 10^{-5}$	1:1,000 "	9.1
5 . . . . .	$50 \times 10^{-5}$	1:2,000 "	9.1
6 . . . . .	$28 \times 10^{-5}$	1:3,600 "	7.4
7 . . . . .	$5 \times 10^{-5}$	1:20,000 "	.

Table I shows the reproduction constant for the ferric chloride solutions. In figure 1 the graphs are plotted and  $K$  is the slope of the curve. The manganese was added as manganous chloride except in solution 7, where it was present with the ferric chloride.

The plants in solution 7 reproduced at first, but rapidly got smaller and became brown-tipped. In two weeks they were shriveled and almost lifeless. Solution 6 produced plants of good size but of somewhat light color; the chlorophyll in all the plants receiving iron as chloride was less dense than in the plants with the citrate. This solution, with its 1 part Mn to 3600 million, has rather less manganese than the standard medium formerly used, in which there was about 1 to 3000 million. The increase of the manganese to give 1 part in 2000 million (solution 5) raised the rate of reproduction; but further increase of manganese had no effect, as previously noted by CLARK and FLY (4) with still larger quantities of manganese.

Table II gives the reproduction constant for the ferric citrate solutions and figure 2 the curves. The manganese was supplied as before, except for solution 14 where it was present with the citrate.

TABLE II  
REPRODUCTION RATE AND MN CONCENTRATION

SOLUTION NO.	IRON SUPPLIED AS FERRIC CITRATE		
	MN PER LITER	APPROXIMATE CONCENTRATION	$K \times 100$
	mg.	p.p.m	
8	$2508 \times 10^{-5}$	1:40 million	11.4
9	$508 \times 10^{-5}$	1:200 "	11.4
10	$258 \times 10^{-5}$	1:400 "	11.4
11	$99 \times 10^{-5}$	1:1,000 "	10.4
12	$53 \times 10^{-5}$	1:2,000 "	9.4
13	$31 \times 10^{-5}$	1:3,200 "	7.2
14	$8 \times 10^{-5}$	1:12,500 "	..

The maximum rate of reproduction is higher than with ferric chloride. The break comes between 1:400 and 1:1000 million. As the manganese content is made smaller the rate of reproduction drops. The plants in solutions 11, 12, and 13 are green and healthy, but are smaller with decreasing content of Mn in the solution. Plants in solution 14, which contained approximately 1 part of manganese to 12,500 million, grew for some days but became very small and most of the fronds died.

In some cases, after the plants had grown for about five weeks with a change of medium twice a week, the change was altered to once a week. It was thought that the speed of reproduction might decrease, especially

where the manganese was low. No noticeable alteration could be found, as will be seen from solution nos. 6 and 11 in figures 1 and 2.

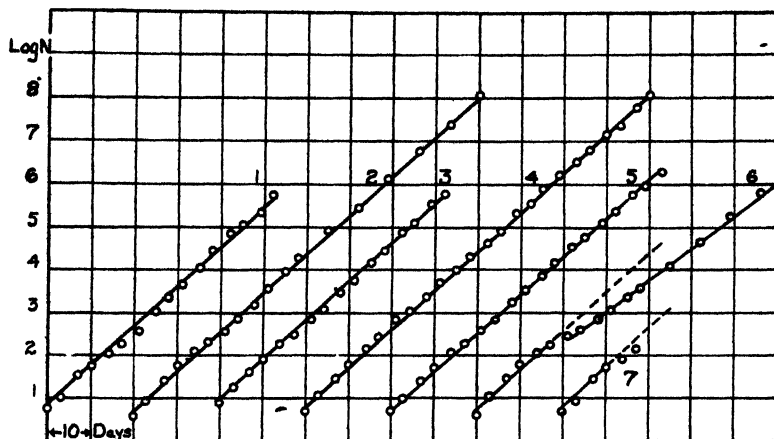


FIG. 1. Reproduction curves with manganese varying. Iron as ferric chloride.

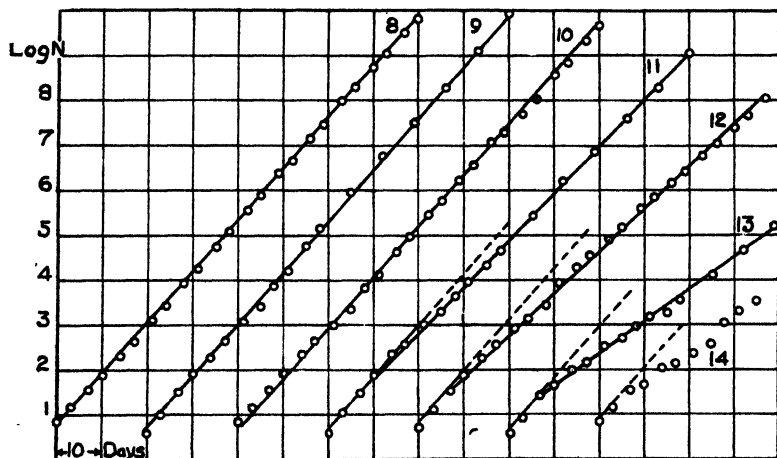


FIG. 2. Reproduction curves with manganese varying. Iron as ferric citrate.

### Conclusions

From these results manganese must be classed as essential for the growth of *Lemna major*. Good growth and healthy plants were produced with a concentration of 1 part of manganese in approximately 3000 million under the conditions of the experiments. With iron supplied as ferric chloride at this concentration, the plants were large but somewhat light colored; with ferric citrate the chlorophyll was dense but the plants were slightly smaller. With increased manganese in the solution the rate of reproduc-

tion rose, but soon reached a maximum, after which further additions of Mn had no effect on the rate.

IOWA STATE COLLEGE  
AMES, IOWA

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HENRI LOUIS DUHAMEL DUMONCEAU  
1700-1782

## BRIEF PAPERS

HENRI LOUIS DUHAMEL DUMONCEAU

(WITH PLATE VIII)

HENRI LOUIS DUHAMEL DUMONCEAU lived at a time when it was possible for a man to be well versed in more than one field of science. He was primarily a physicist, but the desire to apply physics to everyday life has made him better known as an agriculturist, plant physiologist, and plant anatomist. His interest in fruit tree and forest tree growth, fish and fishing, shipbuilding and the methods of sanitation of ocean-going vessels, made him so well known in France that he was made Minister of Marine. His life and works are very briefly reviewed in the *Biographie Universelle* by DU PETIT-THOUARS, and in *Éloge de M. DUHAMEL* by CONDORCET.

DUHAMEL was born in Paris in 1700. He was descended from LOTH DUHAMEL, a nobleman of Holland. CHARLES, the son of LOTH DUHAMEL, came to France about 1400 and settled in Denainvilliers. HENRI LOUIS DUHAMEL's father was ALEXANDER DUHAMEL, Seigneur of Denainvilliers, and his mother was ANNE TROTTIER.

Very little is recorded of his boyhood days. It is known, however, that he made only slight progress in his early studies, except in physics. It is said of him that while he was at the College of Harcourt he retained only one thing, namely, that men in observing nature had created a science called physics.

In order to study this subject he took up lodging close to the Jardin du Roi, the only public institution in Paris at that time where physics was taught. Here he made many friends, some of whom were DUFAY, GEOFFROY ST. HILAIRE, BERNARD JUSSIEU, VAILLANT, and LEMERI. His interest in plant life was aroused by his association with these men, and the study of plants from the point of view of physics became his primary interest. He made such rapid progress in his work that soon he was asked by the Académie des Sciences to solve a difficult problem. A disease reported to be very contagious was attacking the saffron plantations in the Gatinais. Because of its severity the government asked the help of the Académie, and M. DUHAMEL was chosen to make the study. He soon discovered that a fungus in the soil was killing the saffron. As a result of his successful work on this problem he was elected a member of the Académie des Sciences when he was but 28 years of age.

Most of the knowledge developed from the Renaissance to the beginning of the nineteenth century was collected for the glory of the individual investigator and not for the benefit of society. DUHAMEL believed in using

the new truths for the benefit of the country, however, and sought always to better the conditions of the people by his knowledge of physics. His desire to make more truths known prompted him to devote all of his effort toward that end. From the time DUHAMEL was made a member of the Académie des Sciences he devoted nearly all of his time to experimenting and writing on plant physiology. The great services he rendered to agriculture and the science of navigation were a result of this attitude toward knowledge.

He was rather severely upright and modest. As a young man he appeared to be disinterested in people. This and his work kept him from having serious thoughts of a family, and he never married. Except for the time spent in Paris as a student, and later as a member of the Académie and as inspector general of marine, which required some travel, he spent his life with his brother, practicing agriculture.

In 1740, DUHAMEL had become very much interested in meteorology. He began meteorological observations on his land in the Gatinais, and kept detailed records of the weather and its influence upon crop production. Owing to his interest in frost and its effect upon plants, his observations were most intense in the spring and autumn. He attempted to explain the frost hardiness of certain types of fruit trees and the reasons for the lack of hardiness of others. These weather observations, kept throughout the remainder of his life, greatly influenced certain agricultural practices in France.

While he was doing his work in agriculture, plant physiology, and anatomy, LINNAEUS was developing his ideas on systematic botany and laying the foundation for modern nomenclature. STEPHEN HALES had written his "Vegetable Staticks" while DUHAMEL was still a young man, and undoubtedly much of the latter's inspiration and knowledge came from the venerable HALES. By comparison of their works it is obvious that a large part of DUHAMEL's work was compiled and translated from the works of HALES, MARIOTTE, and MALPIGHI. During his lifetime he contributed more than sixty memoirs to the Académie des Sciences, and he published numerous textbooks which were soon translated into other European languages, largely because of the great knowledge of agriculture included. It has been said that DUHAMEL DUMONCEAU's greatest contribution to plant physiology was the bringing together of materials from various sources and giving to the world a summary of the work done in the past.

His work on nutrition is not generally accepted. He believed that the digestion of food occurred in the earth and that the leaves produced the suction required to draw the prepared food material into the roots and stems.

Probably the most valuable of DUHAMEL's work in plant physiology was on the movements of certain plant organs. The periodic movements

of leaves and the curvatures of shoots had already been observed by RAY, who attributed them to changes in temperature. DODART thought that the movements were due to the contractility of fibers on the dry side of the stem. DUHAMEL observed that some of these were heliotropic curvatures caused by light, but that in the case of *Mimosa* leaves the movements took place in darkness as well as in light.

His work on growth followed closely the experiments of HALES, and many of the latter's results were confirmed by new investigations, especially those in which the manner of apical growth in roots was shown. Much of his work on soil, fertilizers, and agricultural crop production was based upon the writings of the English agriculturist, JETHRO TULL. Because of his interest in soils, DUHAMEL made some chemical analyses, and in 1736 he clearly distinguished between the alkalis, potash, and soda.

While DUHAMEL was inspector general of marine he had an opportunity to see the importance of fruit, wheat, and timber to the nation. Owing to his influence, naval construction and sanitary conditions of sea-going vessels were greatly improved. The demand for shipbuilding material caused him to look into the future and to foster tree planting. His interest in forestry, combined with his natural curiosity and desire for useful knowledge, prompted him to study more thoroughly the anatomy and physiology of trees.

From 1755 to 1768 he wrote his best works on vegetable anatomy and physiology, and included practical information on seeding and planting of trees, the growth of wood, its durability, strength, and other physical properties. There are five treatises with a total of nine volumes written on these subjects. The most important of these is "*La physique des arbres*," published in 1758, in two volumes. These two volumes are a complete treatise on plant anatomy and physiology. They are based upon the works of MALPIGHI, GREW, HALES, and BONNET. The numerous figures and plates, numbering fifty-five, are very well made, and many of them are redrawn from the illustrations in HALES' "*Vegetable Staticks*."

"*La physique des arbres*" remained the most complete and most instructive French publication on plant physiology for many decades. It was needed at that time to stimulate further the scientific spirit which was spreading over Europe. It came at the time when PRIESTLEY was beginning his investigations in England and just before INGEN-HOUSZ and LAVOISIER began their famous researches. DUHAMEL's work was a foundation for many of the important physiological investigations. This was especially true in France, before and after the revolution.

It has been said that DUHAMEL had an instinct for truth in his work on plants, but that he lacked the faculty of combination which is so necessary for thorough investigators in plant physiology. He was an uncritical

compiler as compared with MALPIGHI and HALES; and his biographer, DU PETIT-THOUARS, says that he lacked the ability to distinguish between matters of secondary and those of fundamental importance.

At the age of fifty, DUHAMEL was considered one of the most learned men of Europe. His ardor for study never diminished, but he slowly took on the weight of years and was denied the power to carry on the further scientific work which he had planned. He was honored by being made a member of the Royal Society of London and Dean of the Académie des Sciences before his death. He died in Paris on the 13th day of August, 1782, following a stroke of apoplexy. CONDORCET says of him, "His course, useful, glorious, and peaceful, is one of the happiest that the history of science is able to present."—WARREN W. CHASE, *Division of Forestry, University Farm, St. Paul, Minn.*

#### NITROGEN CHANGES IN STORED ALCOHOLIC EXTRACTS OF PLANT TISSUES<sup>1</sup>

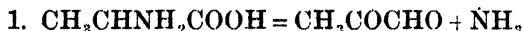
Most phyto-chemists, when desiring to make an analysis of a plant for nitrogen fractions, choose some procedure using fresh tissue. Some workers, however, still make certain nitrogen determinations on alcoholic solutions, and older literature contains references to a considerable number of such determinations. In order to evaluate such work properly, the writer has made a study of the influence of certain factors on stored extracts of various plants, using an aeration method for estimation of ammonia and the Van Slyke apparatus for amino nitrogen.

Such factors as reaction, time of storage, percentage of alcohol, and presence of minerals were considered. Although the type of material influenced the amount of change, in all but one experiment a continually decreasing percentage of amino nitrogen was found, and conversely an increasing percentage of ammonia. In the tests the concentration of alcohol was found to exert little, if any, influence on the final results. The reaction of the solution was probably the most important chemical factor involved, extremes of both acid and alkali inducing greatest changes. Length of storage is perhaps the dominating factor since these changes are gradual. The most extended test covered two and one-half years, and by this time equilibrium seemed to have been reached in the solution. Some few samples stored for a year also seemed to have reached equilibrium, but these were exceptional.

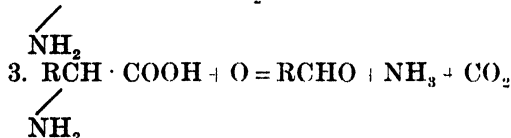
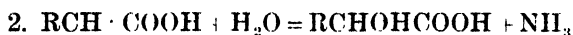
Summarizing the data presented here, it is evident that under all conditions, and with all types of material investigated, progressive changes

<sup>1</sup> Published with the permission of the Director of the Oklahoma Agricultural Experiment Station.

take place in the nitrogenous fractions when plant materials are stored in alcohol. Since this is true, it is desirable to see whether an explanation of the facts recorded can be found. DAKIN and DUDLEY<sup>2</sup> state that under certain conditions in an acid solution, amino acids will undergo decomposition with the formation of ammonia. An example of this is shown in equation 1.



It is also known that under appropriate conditions, amino acids may be deaminized in other ways, two of which follow:



If either of two of these equations (1, 3) should represent the reaction taking place, we should be able to substantiate the fact either by finding an increase in ammonia or an increased reducing power. In a few of the experiments recorded there is enough of an increase in ammonia to account for the decrease in amino nitrogen. Generally, however, the decrease in amino nitrogen is greater than can be accounted for by the increased amount of ammonia. In the case of a synthetic solution, the decrease of amino nitrogen was roughly ten times the increase in ammonia.

It should also be possible to check on the increase in reducing power of the solution if the reaction is such as will form a compound having a free aldehyde group (equations 1 and 3). So far, experiments carried out to detect, if possible, changes in reducing power have yielded conflicting results. In pure solutions of sugars and amino acids a slight decrease has appeared, while in natural extracts a very slight increase was noted. Extensive and painstaking work will be necessary to settle this point, for even if there is an increase it will probably be slight and effective over a long period of time.

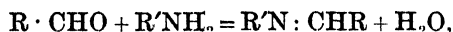
There is considerable published evidence to the effect that sugars and amino acids can combine under various conditions, even in rather dilute solutions. KOSTYTSCHEW and BRILLIANT<sup>3</sup> report that both amino acids and ammonia react with sugars, chiefly those having aldehyde properties. They also state that the reaction is spontaneous in an alkaline medium. WALD-

<sup>2</sup> DAKIN, H. D., and DUDLEY, H. W. The interconversion of alpha amino acids, alpha hydroxy-acids, and alpha ketonic aldehydes. Part II. Jour. Biol. Chem. 15: 127-143. 1913.

<sup>3</sup> KOSTYTSCHEW, S., and BRILLIANT, W. Die Verwandlungen der Aminosäuren in Gegenwart von Zucker. Zeitschr. physiol. Chem. 127: 224-233. 1923.

SCHMIDT-LEITZ and RAÚCHALLES<sup>4</sup> report several experiments on the reaction of simple peptids and glucose. They found a rapid reaction at room temperature between the two compounds in alkaline solutions. Various concentrations, such as 1 mol. of glycyl-glycin to 50 mol. of glucose, gave a 37 per cent. combination. Fructose apparently did not react so readily. BORSOOK and WASTENEYS<sup>5</sup> report that there is a reaction between amino acids, peptones, and glucose. At the same time they state that there is no evidence of a synthetic reaction changing the lower fragments into complex derivatives. Neither did they observe the formation of any ammonia, urea, or like compounds. In one experiment they found a decrease in amino nitrogen in slightly acid solution, although the change was not large.

In view of the fact that there is a greater decrease in amino nitrogen than can be accounted for by supposing deaminization, it is well to consider reactions of this type as an explanation of the results reported here. The general equation may be of this type:



and can explain the decrease in amino nitrogen without any increase in ammonia. While no direct evidence is advanced to prove that this type of reaction actually occurs, still the knowledge that amino acids and glucose can so react lends some credence to the supposition.

It must be borne in mind that the soluble peptids are probably concerned in the indicated reactions as well as amino acids, since no effort was made to remove them before running the analyses. In fact, evidence has been secured, using pure solutions of glucose and amino acids, indicating that much of the change is due to changes in this polypeptid fraction.—JAMES E. WEBSTER, *Oklahoma Agricultural and Mechanical College, Stillwater, Oklahoma.*

## CORRELATION OF SPECIFIC HEAT AND PERCENTAGE OF WATER IN APPLE WOOD<sup>1</sup>

(WITH ONE FIGURE)

The use of calorimetry as a means of measuring the water-holding capacity against freezing in colloidal systems originated with THOENES.<sup>2</sup>

<sup>4</sup> WALDSCHMIDT-LEITZ, E., and RAUCHALLES, G. Zur Spezifität der Peptidasen. II. Vergleich der Peptid-zucker-kondensation mit der Wirkungsweise des Erepsins. Ber. deut. chem. Ges. 61: 645-656. 1928.

<sup>5</sup> BORSOOK, H., and WASTENEYS, H. The interaction of free amino-nitrogen and glucose. Biochem. Jour. 19: 1128-1137. 1925.

<sup>1</sup> Journal paper no. B55 of the Iowa Agricultural Experiment Station.

<sup>2</sup> THOENES, F. Untersuchungen zur Frage der Wasserbindung in Kolloiden und Tierschen Geweben. Biochem. Zeitschr. 157: 174-186. 1925.

ROBINSON<sup>3</sup> is responsible for many improvements in the procedure, and at the present time it is probably the most satisfactory method for determining frozen water in fresh tissue.

Briefly the method consists of accurately measuring the change in temperature of a known quantity of water by the addition of the frozen material. The temperature of the frozen sample is read immediately before it is dropped into the water. The ice formed in the tissue greatly increases the heat required to warm the sample, and if the weight of the material is known the free or frozen water may be calculated from the following equation :

$$X = \frac{(T_3 - T_4) - SW(T_2 + T_4)}{80 - \frac{T_2}{2}}$$

In the equation :

- X = grams of free water
- (T<sub>3</sub> - T<sub>4</sub>) = temperature change in water
- S = specific heat of material
- W = weight of material in gm.
- (T<sub>2</sub> + T<sub>4</sub>) = total temperature change in sample
- T<sub>2</sub> = temperature of sample while frozen
- 80 = calories of heat per gm. of ice.

The equation given is incomplete, in that corrections are omitted for the influence of the apparatus and room temperature upon the temperature change in the water. A detailed discussion of the method is presented by ROBINSON.<sup>3</sup>

The equation requires the use of the specific heat of the material under experimentation. With a number of samples the additional labor involved in making this determination is no small item, since the specific heat of each sample (determined from its duplicate) should be known for accurate results.

A study of the comparative hardiness of fifteen apple varieties at Iowa State College has shown that this additional determination may be eliminated, and the specific heat calculated directly from the water content of the fresh tissue. Since water has a higher heat capacity than any liquid or solid known, it should be an important factor in a measure of the specific heat of any fresh tissue. Figure 1 indicates that water is the determining factor, and that specific heat is almost perfectly correlated with the percentage of water of the tissue.

The position of the regression line was calculated from 65 observations made on current-season apple shoots taken at intervals throughout the period from May to December, 1931. A positive correlation (between

<sup>3</sup> ROBINSON, WM. Relation of hydrophilic colloids to winter hardiness of insects. Colloid Symposium Monograph 5: 199-218. 1927.

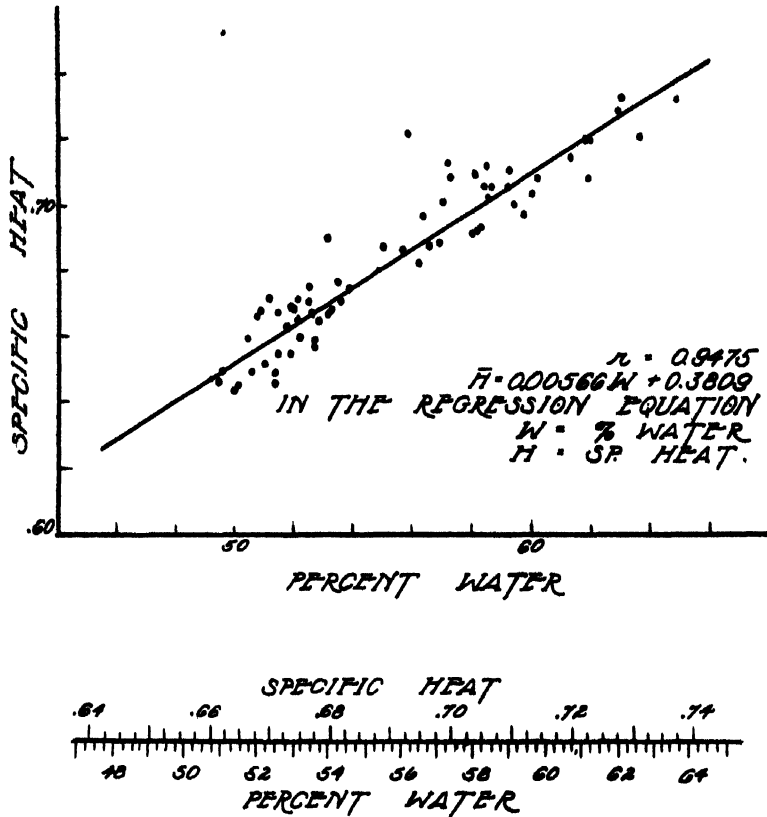


FIG. 1. Relation of specific heat and percentage of water in apple shoots.

specific heat and the percentage of water in the wood) of 0.9475 was obtained in these samples. According to WALLACE and SNEDECOR,<sup>4</sup> a correlation of 0.314 is statistically significant, and the high correlation obtained makes it evident that the specific heat of any sample may be read with more accuracy from the graph than it can be measured from its duplicate. Using the graph, all that is needed to obtain the specific heat of a sample of green apple wood is its percentage of water. In a more convenient form the data may be arranged as shown in the scale below the graph. The value of such an arrangement is obvious when a number of determinations on similar material are to be made.—ARVIL L. STARK, *Iowa Agricultural Experiment Station, Ames, Iowa.*

<sup>4</sup> WALLACE, H. A., and SNEDECOR, G. W. Correlation and machine calculation. Iowa State College Official Pub. 30: no. 4. 1931.

FIELD PROPAGATION OF COTTON BY MEANS OF GRAFTS<sup>1</sup>

Grafting as a method of reproducing cotton asexually first received the attention of the writer in 1930. The results secured from these earlier trials demonstrated the practicability of grafting cotton under greenhouse conditions.<sup>2</sup> The present report gives the results of a grafting experiment conducted during the fall of 1930 to determine the feasibility of grafting cotton plants under field conditions.

The Lone Star variety of cotton was used in this test and the plants had already matured bolls before the grafting was commenced. The saddle-graft method was used on all plants and the individual grafts were made with the minimum lapse of time between cutting the scions and placing them. The stocks remained in the soil with their roots undisturbed in each case.

The field grafting was done on September 18, 19, 21, and 22, under a schedule that provided for work from 1 to 10 P. M. for two of the days, and from 4 to 11 A. M. for the others. A 500-watt lamp was used to illuminate the field for work during darkness. By distributing the grafting throughout the day a wider range of field conditions was introduced. The most important variable was the temperature, which ranged from 68° to 93° F.

After the grafts were completed, they were allowed to remain in the field undisturbed for approximately thirty days. During the development of the unions, the average temperature was 72° F. with an average daily fluctuation of 22° F. In making the final observation, only those grafts that had started stem growth and withstood considerable pressure after the paraffin and twine were removed were considered successful. A total of 220 grafts was made and 87.2 per cent. of them were successful. Also the percentage of successful grafts increased with each additional day's experience. The percentages of successful grafts for September 18, 19, 21, and 22 were 73.3, 88.8, 90.0, and 100.0 respectively. It is quite evident from the results secured that cotton can be grafted successfully under a wide range of field temperatures.—H. E. REA, *Texas Agricultural Experiment Station, Substation no. 5, Temple, Texas.*

<sup>1</sup> Contribution from the Division of Agronomy, Texas Agricultural Experiment Station. Approved by the Director as Technical Contribution no. 228.

<sup>2</sup> REA, H. E. Grafting experiments with cotton. *Plant Physiol.* 6: 193-196. 1931.



## NOTES

**Annual Meeting.**—As the January number of *PLANT PHYSIOLOGY* approaches publication, the ninth annual meeting program is in an advanced stage of preparation. The program committee has worked tirelessly to provide an excellent series of meetings. The members of this committee, of which Dr. JOHN W. SHIVE of the New Jersey Agricultural Experiment Station is chairman, deserve great praise for the work they have done. No one who has not been in charge of the preparation of such a program realizes the amount of detail that must receive attention to insure a successful and satisfactory meeting. Atlantic City will provide a high mark for the emulation of future committees.

**Barnes Award.**—The CHARLES REID BARNES life membership committee, consisting of Dr. W. F. LOEWING, Iowa, chairman; Dr. C. G. DEUBER, Yale; Dr. R. P. HIBBARD, Michigan State College; Dr. E. S. JOHNSTON, Smithsonian Institution; and Dr. R. S. GIRTON, Purdue, has selected Dr. CHARLES F. HOTTES, Professor of Botany at the University of Illinois, as the 1932 life member. There are now eight members who have been honored by the Society in this manner. The award was established at the Kansas City meeting in 1925, the first award being made at Philadelphia in 1926. A double award was made in 1929.

Dr. CHARLES FREDERICK HOTTES was born at Mascoutah, Illinois, July 8, 1870. His undergraduate work was done at the University of Illinois, and his graduate work began in the same institution. He received his bachelor's degree in 1894, and his M.S. in 1895. Later he studied in Europe, and holds the M.A. and Ph.D. degrees from the University of Bonn. The latter degree was conferred in 1901. He has served the University of Illinois since 1895, except for the period he spent in Europe: assistant in botany 1895–1898; instructor 1901–1902; assistant professor 1902–1913; professor of plant physiology since 1913; and head of the Department of Botany since 1928. He has also been consulting plant physiologist for the Department of Agronomy, and is the only member of the Arts and Science faculty to be also a member of the agricultural faculty in the University. Dr. HOTTES has always been an inspiring teacher, and has distinguished himself in the field of cellular physiology, particularly experimental cytology. He has also shown great ingenuity in the designing of apparatus for the control of environment of plants. The Society has been greatly honored by the committee's action.

**Executive Committee.**—The Executive Committee of the American Society of Plant Physiologists for 1932–1933 consists of the following members: Dr. DENNIS R. HOAGLAND, Dr. C. O. APPLEMAN, Dr. W. A. GARDNER, and Dr. W. E. TOTTINGHAM, as present and past officers, and Dr. F. B. CHANDLER and Dr. H. R. KRAYBILL of the Minnesota and Purdue sections respectively.

**Physical Methods.**—The present constitution of this committee is as follows: Dr. EARL S. JOHNSTON, Smithsonian Institution, chairman; Dr. A. L. BAKKE, Iowa State College; Dr. R. B. HARVEY, University of Minnesota; Dr. D. R. HOAGLAND, University of California; and Dr. WILLIAM SEIFRIZ, University of Pennsylvania. It is expected that invitation papers dealing with physical methods of research will be prepared under supervision by the committee, and published in PLANT PHYSIOLOGY from time to time.

**Memorial Committee.**—This committee has charge of all memorial programs. At present it consists of Dr. F. M. ANDREWS, Indiana University, chairman; Dr. CHARLES F. HOTTES, University of Illinois; and Dr. C. O. APPLEMAN, University of Maryland. This year the memorial program commemorated the centennial of the birth of SACHS. The program was participated in by Section G of the A. A. A. S., the Botanical Society of America, the Phytopathological Society of America, and the American Society of Plant Physiologists. On the program, representing three of these organizations, were three outstanding American botanists. Dr. CHARLES E. ALLEN represented Section G of the A. A. A. S., Dr. DOUGLAS H. CAMPBELL represented the Botanical Society of America (paper read by Dr. J. G. PEIRCE), and Dr. RODNEY H. TRUE represented the American Society of Plant Physiologists. This meeting brings to a close the Society's commemorative program for 1932. The committee in charge of this feature did excellent work and deserves much credit for the splendid program.

**Finance Committee.**—The Finance Committee has been reappointed with the same personnel as for the last three years: Dr. CHARLES A. SHULL, University of Chicago, chairman; Dr. WALTER THOMAS, Pennsylvania State College; and Dr. BURTON E. LIVINGSTON, Johns Hopkins University. Any one desiring to contribute, either in a large or a small way, to the several funds operated by the Society is invited to communicate with the chairman, or with the secretary-treasurer, Dr. WRIGHT A. GARDNER, Alabama Polytechnic Institute.

**Support.**—We wish to appeal again to our present members to maintain membership through the financial crisis if at all possible. Or if this is not

possible, we urge that the member try to find some one to take his place on the Society's roll of members. During the past seven years the cost of publishing *PLANT PHYSIOLOGY* has been cautiously increased as the Society enlarged, until each member now pays for less than he receives. As long as there was active purchase of back numbers, this could be done safely. At present this income is greatly reduced, and it is necessary to depend on current dues and subscriptions for the entire cost. If we are to avoid cutting into our reserves, our plans for volume 8 must consider the possibility of reduction in size of the volume unless the current income remains steady. The editors will make such a reduction if necessitated, but as there are many manuscripts on hand, it would mean delayed publication of those already accepted and the necessary rejection of many good manuscripts. It costs well over \$4,000 per year to publish the journal, and maintenance of our membership and subscription lists at their present size is the only means of securing this amount to publish the volume of 1933. *PLANT PHYSIOLOGY* is meeting a real need in the field of scientific journalism. Your help is requested to maintain it in unimpaired efficiency.

**Manuscripts.**—It is necessary to remind our contributors that tables and cuts should be used sparingly. It is unfortunate that these two items make up so large a part of the costs of printing. The editor has noted an increase in the percentage of space given to tables and figures in recent papers. The January and April numbers are overloaded in this regard. Please study manuscripts before submitting them, with regard to the possibility of describing the results other than in tabulated form, and with only an occasional graph or photograph. Papers with one or two tables and a photograph or two are not expensive; but when many full-page tables are included in a single paper, the costs become prohibitive. If writers will maintain a sense of responsibility toward the costs of printing, knowing that they can never pay to the journal anything but a fraction of the costs of publishing their own writings, it will be very helpful. If *PLANT PHYSIOLOGY* were heavily endowed, of course, colored plates could be included and all the tables and cuts required for extensive presentation of work could be accommodated. It seems better at present, however, to work on the theory that good research can be done without overloading the expensive features of publication.

The editor desires to state that it is no longer our policy to accept carbon copies of manuscripts; an original typewritten copy must be supplied.

**Minnesota Section.**—The Minnesota Section of the American Society of Plant Physiologists has arranged its program for the year 1932-1933 as follows:

Nov.	1, 1932, Winter hardiness in plants.	DR. S. T. DEXTER
Dec.	13, 1932, The effects of certain toxic substances on the catalase activity of plants.	RAYMOND LANDON WARREN CHASE
Jan.	3, 1933, Gases in trees.	ALFRED VOGELE
Feb.	7, 1933, Factors affecting lycopin formation in plants.	DR. HARDY L. SHIRLEY
March	7, 1933, Drought resistance in forest vegetation.	DR. HENRY SCHMITZ ERNEST ANGELO
April	4, 1933, Recent developments in the theory of the toxic action of wood preservatives.	
May	9, 1933, Hardiness in strawberries.	

Members of the Society visiting the Twin Cities are invited to meetings of the local section.

**Statistical Methods.**—The fourth edition of R. A. FISHER's monograph, *Statistical Methods for Research Workers*, has been received. The third edition was issued in 1930 (*PLANT PHYSIOL.* 5: 440. 1930). This work is one of the best sources of information available for the biometrician. It is designed for use with such numbers of data as are usually obtainable in biological work. The main changes in the fourth edition are as follows: An additional section on the analysis of covariance has been added at the close of Chapter VIII, on the Analysis of variance; and the part played by the covariance has been emphasized in chapters to which this discussion is pertinent, mainly Chapter V. There are a number of changes also in Chapter III, on Distributions, the appendix of which has been entirely rewritten. There are no other changes of consequence. The book is published by Oliver and Boyd, 33 Paternoster Row, London; and the price is 15 shillings net.

# PLANT PHYSIOLOGY

APRIL, 1933

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## SAP HYDRAULICS

EDWIN DESHLER WOODHOUSE

(WITH ELEVEN FIGURES)

### Introduction

In animals, the chief function of gas exchange in the lungs is to supply oxygen for, and to remove the products of respiration, whereas in plants the chief function of gas exchange in the leaves is to supply carbon dioxide for photosynthesis. Since the protoplasm of the plant or animal body contains a high percentage of water, it is inevitable that both of these processes are accompanied by a loss of water in the form of gas, *i.e.*, water vapor. However, since there is seven hundred times as much oxygen as carbon dioxide in the air (96), which is the normal environment of plants and animals, the latter can obtain that which they need from a smaller volume of air than can plants. Consequently, being necessarily exposed to a larger volume of air, a greater amount of water is evaporated from plants than from animals, and that which is of minor importance in animal physiology becomes one of the major problems of plant physiology.

To replenish the enormous water loss (19, 74) accompanying gas exchange in the leaves necessitates the movement of water through the plant body from the roots to the leaves. Aside from preventing the desiccation of the plant, this water movement and loss may be of minor benefit to the plant (110, 9, 54, 28, 88) in its life processes; nevertheless, it is of sufficient magnitude to arouse the interest of the observer. The warm dry air prevalent during summer in California provides a situation particularly favorable for the observation of these phenomena. If any one phase be studied alone, without due consideration of its relationships to plant physiology as a whole, erroneous conclusions may be reached such as those implied by references in the literature (93, 27) to the transpiring "power" of plants. Consequently, the writer's observations of the transpiration stream have included studies of the entrance of the water into the roots, the passage of that water through the plant, and its exit through the leaves. This inclu-

sive subject I have termed "sap hydraulics," and the present study is the continuation of a previous one bearing the same title (109).

COPELAND (22) gives a review of the early literature on this subject, followed by a bibliography including 174 titles. BURGERSTEIN (20) presents a bibliography with nearly 1,200 titles up to April, 1925. Although but a portion of the total available, the writer has examined an additional hundred pertinent references appearing since that date. MILLER (69) also presents excellent bibliographies. It is obvious that any attempt at a complete review of the literature on this subject would require a monograph.

A consideration of plant anatomy and physiology as a background for sap hydraulics shows that the transpiration stream moves through a system of vessels, the walls of which are wet by water continuous with that which it is conducting. This xylem is surrounded on all sides and terminally by living cells. Conduction of nutrient materials takes place through the phloem by diffusion through the protoplasm, particularly of the sieve tubes, aided by protoplasmic streaming; or by diffusion through the cell walls, aided by concentration gradients and mass flow as recently proposed by CRAFTS (27); or by a combination of the two.

### 1. Entrance of water into roots

The region of entrance of water into the root has been determined by COUPIN (24, 25, 26), PRIESTLEY *et al.* (77, 78, 79, 80, 81, 82, 90, 91) and POPESCO (76). They are in agreement that the region of maximum absorption under conditions of plentiful water supply lies below the region of root hairs and that the latter are not indispensable, although PRIESTLEY points out that, under conditions of deficient soil moisture, they greatly increase the surface area of the root in contact with the soil. Since it is now known (21, 49, 57, 106) that there is but little movement of moisture in the soil under such conditions, and that the roots have to grow to it, the significance of PRIESTLEY's observation is readily appreciated.

The upper limit of the absorptive area is determined by the deposition of waxy materials in the cell walls of the endodermis. The lower limit may be the terminal embryonic region. PRIESTLEY's observation that this region offers a continuity with the protoplasm of the endodermis is morphologically correct; but his contention that no water is absorbed by this region is based upon observations, the insufficiency of which is shown by his later work.

In his earlier work PRIESTLEY made use of dyes in his determinations. He now emphasizes his agreement with other workers (4, 5, 14) that water entry and salt entry are distinct processes, using, as evidence, some of the very dyes from both the acid and basic groups which he had used in attempting to delimit the zone of water entry.

The writer has performed experiments with four of the dyes used by him, namely, methyl green, acid green, malachite green, and neutral red. These dyes were added to dilute lithium nitrate solution as a medium for water cultures of corn, sunflower, buckeye, and willow plants. In every instance the lithium passed up the stem as shown by spectroscopic test, while not the slightest trace of the dye could be found. These experiments merely emphasize the independence of movement of the water and the dye dissolved in the water, as elucidated by SACHS (85). The same conclusion

*Maturation of Xylem  
IN ROOTS  
showing comparison to development  
of other tissues.*

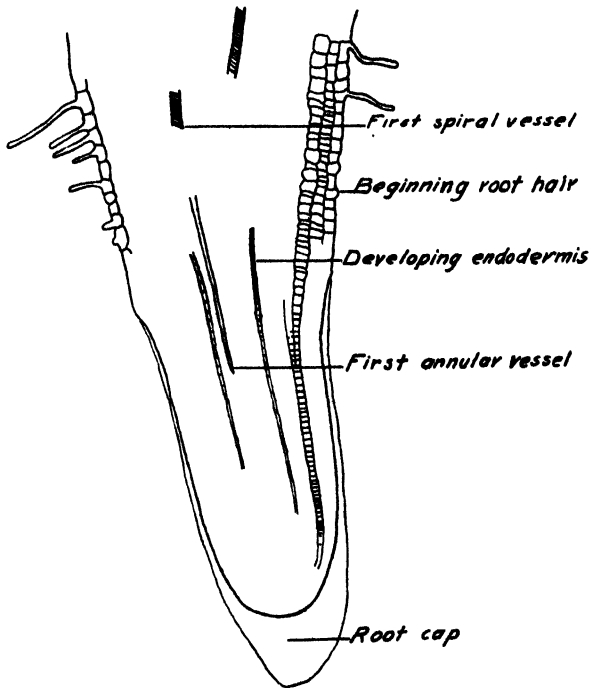


FIG. 1. Longitudinal section of *Cosmos* sp. root tip showing relationship of maturing xylem vessels to other tissues.  $\times 70$ .

was reached by the use of a fifth of these dyes, eosin, on potted plants of eucalyptus. The eosin diffused into the roots so slowly, even in 24 hours, that it did not appear in the xylem at all.

POPESCO's conclusions are therefore to be preferred, namely, that some water may be absorbed by the embryonic region of the root for growth at that point. Absorption of water for the vessels takes place between the

level at which the differentiation of vessels begins up to the level at which the endodermis is suberized, with a maximum at the lower limit of the region. Since spiral tracheae can be found within 1 mm. of the root tip (fig. 1), and COUPIN immersed his root tips 2 to 3 mm., his conclusions are not in discord with those of POPESCO.

Considering next the mechanism of water entry: under conditions of rapid transpiration or deficient soil moisture, where the water in the vessels is under tension, that tension may be transmitted through the tissues of the root to the soil moisture, drawing the water into the root without the intervention of any osmotic phenomena. The fact that passageway cells in the endodermis opposite the xylem points are at the level of developed root hairs is of significance.

Under conditions of plentiful water supply, PRIESTLEY (*loc. cit.*) explains water entry and root pressure by an osmotic gradient between the soil solution and the inside of the endodermis, set up by the liberation of solutes when the cell membranes of the differentiating xylem break down and become permeable. This would necessitate active growth for the manifestation of root pressure, and is in accord with the observations presented herewith. However, in the case of *Colocasia* at least (FLOOD 38), this would necessitate the assumption of resorption of the solutes. After the protest of BLACKMAN (11), PRIESTLEY presented evidence to justify this assumption. An alternative mechanism will be presented which does not necessitate this assumption and which is in better accord with root anatomy.

## 2. Guttation experiments showing correlation between active growth and root pressure

In a previous study on sap hydraulics, I have shown that guttation is not due to any local glandular action, but rather to hydrostatic pressure arising within the roots. This is in accord with the observations of FLOOD (38) and LEPESCHKIN (55).

In the present study use has been made of a large abandoned cistern located under the experimental glass-house of the Stanford Botany department. The natural environment provided by this cistern is such as to make artificial control of conditions unnecessary. Seepage water affords a constant and practically saturated atmosphere. The temperature in the absence of a light is constant, over a considerable time.

A series of five experiments was run on seedling wheat and barley, and one on nasturtium. It was necessary to use powdered sulphur on the surface of the soil in order to keep down the growth of fungi.

EXPERIMENT 1, started February 11, 1931; temperature 20° C.; no light.

A pot of seedling wheat was placed in the cistern. Water of guttation was wiped off the leaves for the next five days. There was no guttation on

the sixth day. Placing the pot in a pan of water produced no guttation, showing that the amount of water at the roots was not a limiting factor. On the seventh day the pot was placed under a bell jar and removed to the basement, thereby subjecting the plants to a drop in temperature of  $5.5^{\circ}$  C. This produced but very slight guttation.

By this time the plants were pale green in color, due to growth in the dark. On the evening of the eighth day, the bell jar was placed under a strong light. The following morning there was some injury from the heat of the light and the plants were returned to the cool basement. The next morning there was copious guttation, even on the tips of leaves completely desiccated from heat injury.

EXPERIMENT 2, started February 11, 1931; temperature  $20^{\circ}$  C.; no light.

Wheat seeds were germinated in a pot of soil under a bell jar with an excess of water. There was slight guttation on the tips of the seedlings. On the third day following germination, the lid was removed from the cistern for several hours, permitting sunlight to enter. The following day all of the seedlings showed marked elongation and active guttation.

EXPERIMENT 3, started April 27, 1931; temperature  $21^{\circ}$  C.; no light.

A pot of seedling wheat was placed under a bell jar, with an excess of water. Guttation had ceased by the seventh day. The plants were again a pale green.

At 10:30 on the morning of the eighth day, a 200-watt nitrogen-filled Mazda lamp was suspended about a meter above the bell jar and turned on for just five minutes. Four hours later, guttation had been resumed by one of the plants. The light was then turned on for a full hour, producing copious guttation against a rise of  $0.5^{\circ}$  in temperature. This observation of guttation under a rising temperature led to the following experiment.

EXPERIMENT 4, started May 11, 1931; temperature  $21^{\circ}$  C.

A pot of barley seedlings was placed under a bell jar with an excess of water at 11:00 A. M. At 1:30 P. M. guttation was visible and the 200-watt light was turned on. Four hours later the temperature had risen  $1^{\circ}$ . All of the plants were guttating freely.

By the fifth day the temperature had risen to  $23.5^{\circ}$  C. The plants had been guttating continuously under the rising temperature. On the sixth day the temperature had risen to  $24^{\circ}$  C. and guttation had ceased, probably owing to decreased humidity at the higher temperature. That night the light was turned off and the cover removed from the cistern. This lowered the temperature again to  $21^{\circ}$  C. The following morning the light was turned on once more and the plants resumed guttation. In this manner the plants were made to guttate almost continuously for two weeks.

EXPERIMENT 5, started May 25, 1931; temperature  $21^{\circ}$  C.

A pot of barley seedlings which were grown in a dark room until guttation had ceased, even with an excess of water at the roots, was placed in the cistern under a bell jar with an excess of water. These seedlings had made good growth and showed guttation on two or three blades only when placed under the bell jar. The light was then turned on, but no additional guttation could be produced. Even placing the bell jar with the plants in an ice-box did not produce guttation.

This experiment was repeated with a mature water culture of wheat which had developed a head. No guttation could be produced by illumination, decreased temperature, or increased humidity of the atmosphere.

**EXPERIMENT 6.** A potted nasturtium which had ceased active growth was placed under a bell jar with an excess of water. The nasturtium is a standard plant for experiments on guttation; nevertheless this plant did not guttate even though the conditions were such as to cause condensation of water in the bell jar. In another instance such a plant was caused to resume guttation by repotting and allowing new root growth to occur.

### CONCLUSIONS

These experiments show that even under conditions of lowered temperature, high relative humidity, and an excess of water in the soil—all of which are favorable to guttation,—plants will not show this phenomenon unless actively growing. If actively growing, then, with an adequate water supply and a high relative humidity, plants will continue to guttate even in a rising temperature.

Since guttation is a manifestation of root pressure, the latter is produced only under conditions of active growth.

### 3. Alternative hypothesis for water entry into roots in absence of tension forces

Knowing that root pressure is developed only under conditions of active growth, a review of root anatomy leads to the following explanation of water entry in the absence of tension forces from transpiration, extending the mechanism of PFEFFER (75), BLACKMAN (11), and MÜNCH (71).

The root is terminated by a region of actively growing embryonic cells. In the process of growth, cells absorb large amounts of water. Therefore the cells of the root tip will absorb water directly from the soil for this purpose; the cells of the root cap beyond the embryonic region have no easier means of obtaining it.

The enlarging cells above the embryonic region may also absorb water for growth directly from the soil. In addition to water, however, these cells and the terminal meristem itself need large amounts of nutrients which are brought in solution through the phloem. These solutes, by metabolism,

will be incorporated into the substance of the cell. The excess solvent, after providing water for growth, will be expelled by the path of least resistance. The active growing point is on the inside of the root and the maturing vessels are also on the inside of the root; consequently the excess water will be expelled into the vessels of the xylem. The endodermal sheath will tend to prevent the escape of any water.

In the region of the root where maturation is taking place, the phloem occupies as much as nine-tenths of the circumference of the stele adjacent to the endodermis (fig. 2). The phloem is carrying, either in solution or

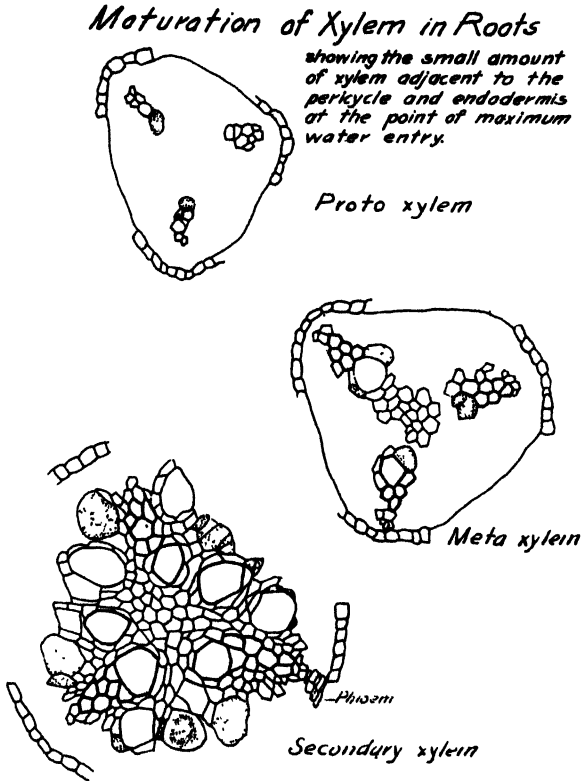


FIG. 2. Transverse section of stele of *Salix lesiolepis* root showing small amount of xylem adjacent to pericycle and endodermis at point of maximum water entry where primary xylem elements are maturing.  $\times 150$ .

chemical combination, a higher concentration of nutrients (or in other words, a lower concentration of water) than the adjacent tissues. Consequently it is natural that water should be drawn through the endodermis into the phloem. Then, following the process of growth in adjacent maturing cells, or those nearer the growing point, that same water will be expelled

into the xylem as the path of least resistance. Small amounts of solutes may be carried with the water, varying with the protoplasm of the plant in question. In this manner a hydrostatic pressure may be created, giving rise to guttation of water containing only traces of solutes and without invoking any mechanism for resorption.

After tension is created under rapid transpiration, water will be drawn directly into the xylem through the passage-way cells in the endodermis at the xylem points.

This explanation would seem to be in better accord with root anatomy than is that offered by PRIESTLEY.

#### 4. Movement of water through vessels

The foundations for sap hydraulics were laid by HALES (41) in 1727. He first called attention to the magnitude of transpiration. He also gave "proof . . . of the serviceableness of the leaves in drawing up the sap" through "perspiration, by the action of warmth" and "the great force with which plants . . . imbibe moisture up their capillary sap vessels," . . . "also of the sap's freely either ascending or descending, as it shall happen to be drawn by the perspiring leaves." "And that plants can plentifully imbibe moisture through their stems and leaves as well as perspire it."

When it was found that capillarity alone was inadequate to account for movement through the xylem, other mechanisms were invoked, such as the alleged lifting power of a Jaminian (46) chain of air bubbles, or the pumping effect of living cells, advocated even today by BOSE (15, 16, 17).

Although living cells in the leaves may be necessary for transpiration, it has been shown that the actual movement of the water through the xylem vessels constitutes a problem in hydraulics. That living cells along the conducting tract, although they may be of value, are not a necessity, is brought out by the work of STRASBURGER (100, 101), OVERTON (73), RENNER (84), SCHMUCKER (89), and MACDOUGAL, OVERTON, and SMITH (66).

The simple physical nature of the problem is further brought out by the experiments on reverse flow and absorption by leaves performed by numerous investigators, including HALES (41), DANDENO (30), WETZEL (108), ARNDT (1), and HARVEY (43). The application of hydraulics is further developed by the correlation of movement through the vessels with POISEUILLE'S (48) formula for capillary tubes made by EWART (37), and the even closer correlation between rate of flow and vessel diameter for different sizes of vessels made by FURR (39).

The search for some simple physical mechanism has revealed but two which have attained general favor with botanists: the imbibition theory of SACHS and the tensile column theory of DIXON and ASKENASY.

SACHS (85, 86, 87), following UNGER (104), conceived of the sap as imbibed in the cell walls, even as water of crystallization is bound in a crystal. In such circumstances the weight of the water is eliminated as a factor in sap hydraulics. Because SACHS insisted that imbibition was a phenomenon distinct from capillarity, COPELAND (22) concluded that, "This robbed the imbibition theory of its foundation in established physics." Both SACHS and COPELAND were correct. LANGMUIR (52), followed by MCBAIN *et al.* (58) and HARDY (42), had not yet developed their theory of sorption now accepted in established physics and confirming SACHS' ideas. It was not because of this, however, but because he thought that the water moved only through the walls of the vessels, that his theory was displaced. Plugging experiments showed that the water moved, for the most part, through the lumen of the vessels.

DIXON (31, 32, 34, 35) and ASKENASY (2, 3) independently developed a theory of sap hydraulics which had been earlier suggested as a possibility, as related by ASKENASY. They postulate tensile stress generated in the sap at the mesophyll cells of the leaves, transmitted the length of the stem through the cohesive force of water, made possible "only when it is adhering completely to a rigid envelope which confers on the liquid a pseudo-rigidity."

That tension forces exist in the trunks of trees there can be no doubt. That they may amount to several atmospheres is possible. That they are not usually sufficient even to balance the atmosphere and produce a zero pressure is shown by the manometer results obtained by JONES *et al.* (47), EWART (37), REINDERS (83), quoted by OVERTON (73) and PRIESTLEY *et al.* (79), COPELAND (23), HUBER (44), and MACDOUGAL (64). These works instance greater tensions found at lower levels, which is the reverse of that which should be found in a tensile column. Although he estimated higher tensions, NORDHAUSEN (72) reached the same conclusion. Also, a careful examination of MACDOUGAL's figures suggests that higher tensions frequently go with atmospheric conditions favorable to the tree, causing a reduction in transpiration. Such results have caused most of these investigators to question the tensile column theory.

##### 5. Experiments showing need of modifying tensile column theory

In an experiment with *Pittosporum undulatum*, PEIRCE and his students obtained after several hours a rise of 49 cm. of mercury, owing to the tension set up by transpiration. Air then appeared at the lower end of the branch, and the column of mercury fell. This was interpreted as an indication that the xylem walls permit air to penetrate before such tensions are created as would be required in tall trees, under the DIXON-ASKENASY hypothesis. As

a result of this experience PEIRCE expressed his doubts as to the adequacy of the tensile column theory at the Pasadena meeting in 1931.

Working with fragments of xylem from conifers, BAILEY (8) found that "the surface tension of sap in the pit membranes of various conifers can be overcome by pressures of less than three atmospheres," and consequently expressed the same doubt.

COPELAND (22) reports similar experiments by a number of investigators, all of whom arrived at the same conclusion.

Although the procedure is not new, a number of such experiments were performed with apparatus as illustrated in figure 3, and results obtained

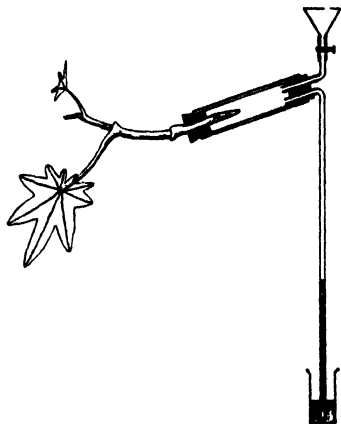


FIG. 3. Apparatus for demonstrating suction tension from transpiration.

which make a significant addition to those already reported.

With *Ricinus communis*, on June 12, under outdoor summer weather, in five minutes a column of mercury was obtained nearly equal to that obtained in the laboratory in a number of hours. The tension was probably greater in the xylem vessels than the 46 cm. of mercury indicated, since the latter was the net result of the water tension and the force with which the air was entering. Also there must have been internal injury from the air passing through the xylem where ordinarily it would have entered and become static. This experiment was repeated on July 3, a day on which the local maximum temperature record of 26 years' standing was officially exceeded. The curves showing the rise of the mercury plotted against time, together with the temperature, relative humidity (from MARVIN's tables, 67), and evaporation<sup>1</sup> from a Livingston standard white porous cup atmometer, are shown in figure 4. On an excessively hot, dry day, when we should expect to find a greater tension exhibited by the plant, it is unable to produce the results

<sup>1</sup> Courtesy of Mr. DELZIE DEMAREE.

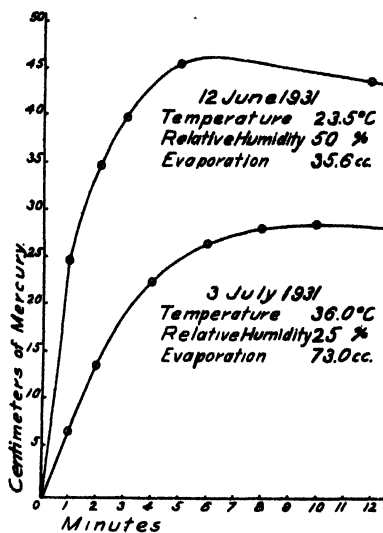


FIG. 4. Record of suction tensions generated by *Ricinus communis* under widely different atmospheric conditions.

obtainable on a more favorable day. MACDOUGAL's work, previously mentioned, was suggestive of the same thing.

Since there is a higher rate of transpiration on a day when smaller tensile forces are exhibited, some other factors must enter into a consideration of sap hydraulics.

The following experiment is suggestive of an explanation for the mechanism of sap hydraulics. In attempting to demonstrate tensile forces in water, a glass U-tube, 14 m. in height, was constructed in a stair-well of the Botany building at Stanford University. The tube was approximately 1.75 mm. inside diameter, and sections were joined with a sleeve of larger tubing held in place by DeKhotinsky cement. At the bottom, as an integral of the tube, was a reservoir with a connection to a second bottle, so that the tube could be filled from the bottom by pressure on the latter, thereby eliminating air bubbles. The top of one arm of the U-tube was connected by a stopcock to a vacuum system. It was hoped that the velocity of the water, descending in one arm of the tube and rising in the other, combined with its cohesive properties, would carry it to an elevation greater than the barometric column. Unlike ASKENASY (3) and THUT (102), under the conditions of this experiment no tensile column was obtained. The water entered the vacuum chamber in spurts, and numerous bubbles formed in the column.

With the aid of a steel piano wire, a thread of white darning cotton (J. & P. Coats 4 ends of 2 ply) was run through the ascending arm of the

**U-tube and the experiment repeated.** Although the rate of flow was reduced, no bubbles appeared and the flow into the vacuum chamber was uniform, emphasizing the importance of the wettable wall to which Dixon has called attention.

The vacuum chamber was then disconnected and the rate of downward flow due to gravity was measured through the tube with the thread. The results are plotted in figure 5. As the height of the hydrostatic column

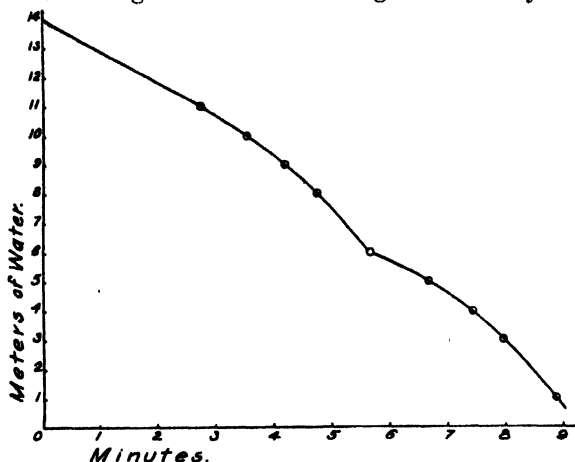


FIG. 5. Downward flow due to gravity, of water through a 1.75 mm. glass tube containing a thread of darning cotton.

decreased the rate of flow increased, showing sorption by the cellulose of the cotton to have been a more important factor than the attraction of gravity. The break in the curve at the height of 6 m. is probably due to some irregularity in the diameter of the tube. Repetition gave duplication.

A cross-section of this thread, when imbedded in paraffin within the tube, occupied 30 per cent. of the lumen of the tube. Planimeter measurements on camera lucida drawings of xylem vessels from twelve or more different plants show that the wall material, considering the middle lamella as the boundary of the lumen, occupies anywhere from 15 to 75 per cent. thereof, the proportion being higher in smaller vessels. These comparisons are presented in figure 6.

This experiment shows that under conditions simulating a xylem vessel, sorption can aid in supporting the weight of the water. In a xylem vessel this would be even more marked, so that any hydrostatic pressure in the tree might be eliminated.

## 6. Combined tensile fluid-imbibition theory

The forces controlling water movement in the plant are those of osmosis, sorption, or imbibition by cellulose walls and protoplasm, and the cohesive

forces in water itself. A balance is maintained among these forces. SHULL (95), in considering this balance, calls attention to the fact that the force of cohesion in water is less "than the adhesion or imbibitional attractions between water and wall substance and protoplasm." VINES (107) had previously offered a similar opinion. Because LECLERC DU SABLON (54) over-emphasized osmosis by living cells in the maintenance of this balance, he

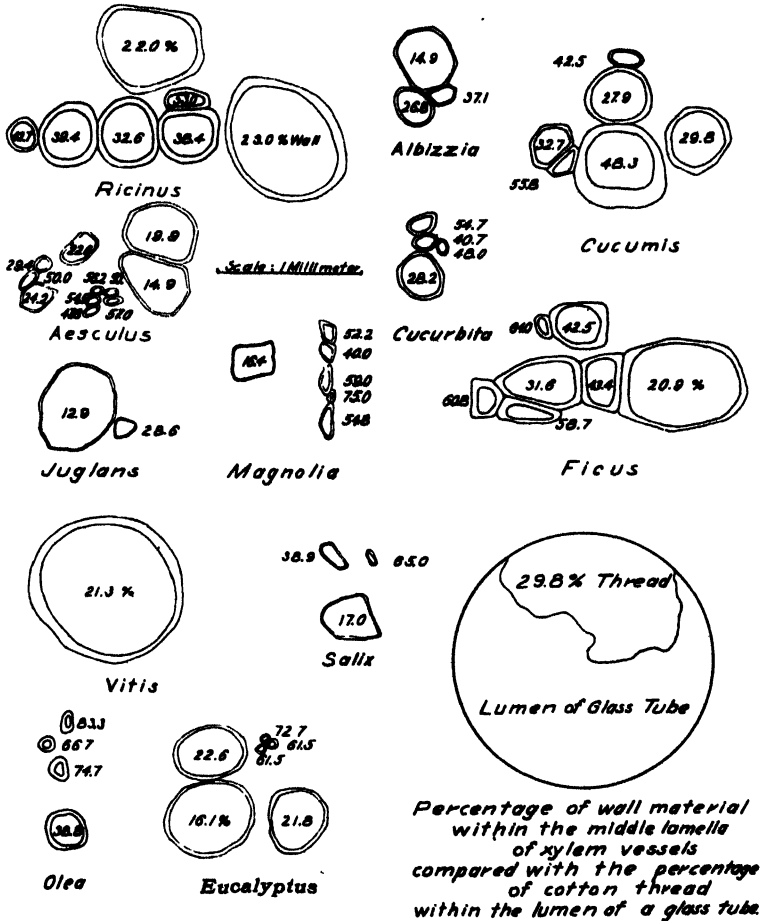


FIG. 6. Percentage of wall material within the middle lamella of xylem vessels compared with percentage of cotton thread within the lumen of a glass tube.

has been classed as a vitalist. His insistence that "la tige supporte seulement le poids de l'ensemble du système" is overlooked.

These observations need not detract from the contribution which DIXON and ASKENASY have made to sap hydraulics. On the other hand, their contentions should not be permitted to detract from, nor overshadow, the con-

tributions of SACHS. The modern conception of sorption as given by LANGMUIR (52), and developed by HARDY (42) and MCBAIN and DAVIES (58), weld these two theories together into a harmonious whole which can satisfactorily explain the mechanism of sap hydraulics. LANGMUIR found that sorbed gases are held in a monomolecular film. In the case of liquids, HARDY, MCBAIN, and DAVIES found that there are oriented chains of molecules attached to the monomolecular films and extending the influence of sorption to measurable microscopic distances. The diameter of xylem vessels, which always contain water, as determined by BODE (12), agrees with these measurements. Thus it is logical to conclude that the "wires of water" are moved by tensile stress, but supported by sorption.

If to this we add the rôle of metabolic water in repairing broken columns in the xylem vessels, overlooked by BABCOCK (7) in his consideration of the subject but developed by MÜNCH (71), we shall have removed LIVINGSTON'S (57) tentative supposition "that if the taut water mass is ever broken in a vessel segment, it is never replaced." By this addition, the theory of sap hydraulics approaches completion. Since MACDOUGAL (59, 60, 61, 63) has shown by dendrographic records that growth in trees takes place at night, such repairs would take place at night, also.

DIXON (35) also suggests a zymogenetic secretive function of living cells, to prevent the vessels from clogging.

### 7. Activating forces in the leaf

VAN TIEGHEM (103) conceived of transpiration as "chlorovaporization," a by-product of photosynthesis. From a study of green and etiolated plants, WOODS (110) concluded that this process was purely evaporation. "It is evident, therefore, that so-called 'transpiration' is not something which protoplasm *does*, but something which it *resists*." More recently, IWANOFF and THIELMANN (45) showed conclusively that transpiration is not chlorovaporization. They found from 17 to 60 per cent. increase in transpiration on changing from the red-yellow or photosynthetic zone of the spectrum to the blue-violet zone.

DIXON (31, 32, 33, 34, 35) has consistently maintained that transpiration is due primarily to secretory action in the cells of the leaves. In the previous study, I showed that water movement in the vessels, in the absence of transpiration, was due to water deficit in cells along the vessels rather than to secretory action; and that DIXON in his experiments did not allow time for a water equilibrium to be established. The comparatively humid climate under which he worked did not sufficiently emphasize the rather long period of time necessary. Further evidence was also presented against secretion through experiments on guttation. This criticism of DIXON has been confirmed by the work of SMITH, DUSTMAN, and SHULL (97).

Although designed for another purpose, one of the experiments of BODENBERG (13) with lithium nitrate on *Salix* offers similar evidence.

The activating force for the transpiration stream most frequently found in current literature is the evaporation of water from the menisci of sub-microscopic pores in the walls of the mesophyll cells of the leaves (62, 66, 69). SHULL (95, 97) gives a detailed presentation of the propagation of this force into the cell and its conversion into imbibitional and osmotic forces.

Much work has been done by URSPRUNG (105) and others (10, 70) toward evaluating osmotic pressure and suction tension. This work distinguishes between the suction tension of the cell under normal conditions, due to the net result of turgor pressure outward and wall pressure inward, and the suction tension of the cell under incipient plasmolysis, due entirely to the osmotic pressure of the cell contents. This work does not distinguish between the suction tension or pressure due to osmotic pressure developed by the cell sap and imbibition pressure or tension developed by the protoplasm itself. Of course these three factors of osmosis by the cell sap, imbibition by the cell protoplasm, and elastic tension by the cell wall are constantly undergoing change in a dynamic system such as a living plant cell.

The work of LIVINGSTON (56), SHREVE (92, 94), BOSWELL (18), DUNN *et al.* (36), MEYER (68), and others (see 6 and 40), has developed the importance of the latter factor; while KUNKEL (51) and LAPICQUE (53) would abandon osmosis entirely. In my previous study, on the basis of theoretical physics, evaporation against the greater attraction of large organic molecules constituting protoplasm was suggested as an alternative force for activating the ascent of sap, or, in other words, the force of imbibition. "Consequently in this case, also, it would take more than 536 calories to evaporate one gram of water, the difference being a measure of the work energy available for lifting the tensile columns." The difference referred to is the heat of imbibition readily demonstrable in a Dewar flask.

That evaporation against the force of imbibition by the protoplasm may be the major factor in activating the transpiration stream is indicated by an observation recently made upon some shrubbery growing on the Stanford campus. Just before the first week in July, 1931, a mass planting of *Choisya ternata* and another of *Coprosma baueri* were pruned down to a uniform height, exposing the shade leaves. This was followed by a period of record-breaking, hot, dry weather (fig. 4). The shade leaves were desiccated, while other leaves upon the same plant, but previously exposed to the sun, were uninjured. Since SPONSLER (98, 99), by X-ray crystal analysis methods, has shown a remarkable uniformity to the molecular structure of cellulose, it is difficult to conceive of any difference in the submicroscopic

pores of the cell walls; and the tension exerted upon the water from evaporation in these pores would be the same in both cases. In the case of the shade leaves, this tension was not sufficiently transmitted through the protoplasm as imbibition pressure to provide the tissues with adequate water. In the hardier sun leaves, the protoplasm was able by imbibition to obtain and keep sufficient water to prevent desiccation.

No adequate correlation has been found (MILLER 69) which could serve as an explanation between the rate of transpiration and the morphological differences between the sun and the shade leaves. This does not necessarily mean that more water was evaporated from the sun leaves, although ZALENSKII (111) has reported such results. OVERTON (73), in his experiments with *Cyperus*, found an increased rate of evaporation from leaves which had been killed. However, this is merely the process of desiccation rather than of transpiration, considered as a part of sap hydraulics; for OVERTON goes on to say, "the percentage of water contained in the plant is very much below that in plants which have not been killed." PEIRCE (74) observed a similar phenomenon in leaves injured by sulphur dioxide.

SHREVE (94) found that "transpiring power" was inversely proportional to the capacity of leaves to imbibe water. On this basis there would be less transpiration from the sun leaves than from the shade leaves, which was indicated by the observations on *Choisya* and *Coprosma* just given. These opposite points of view are reconciled by the work of KISSELEW (50), who found that more water is transpired from sun leaves than shade leaves; but that in the case of *Syringa vulgaris* and *Caragana arborescens*, under critical conditions, the sun leaves were better able to resist the transpirational loss than were the shade leaves.

Since any osmotic tensions must be transmitted through the protoplasm, the imbibition power of the protoplasm becomes the determining factor in the activation of sap hydraulics.

### 8. Application of tensile fluid-imbibition theory

MACDOUGAL *et al.* (65, 66) have found a very marked zonation in the conducting area in the xylem of each annual ring for several different trees. "In the pine the stream moves through practically all parts of each annual ring; in the willow it moves through the late summer wood only of each annual layer; in the alder through the early spring wood only; in the walnut it moves through the early spring wood and late summer wood of such annual layers as have not been transformed into heart-wood."

When first reported, this zonation was attributed to such anatomical features as perforations in the pit membranes, tyloses, and apical connections with the transpiratory systems in the leaves. In the latter paper it is "ascribed to gases in definite portions of each annual layer." No explana-

tion is offered to account for this latter phenomenon. My earlier study offered in explanation the hypothesis that "the transpiration stream flows in the largest tracheae in which a tensile column can be maintained in the environment of the moment."

That the rate of flow is not the same in all vessels is readily observable through the horizontal microscope, using a translucent stem and a dye such as eosin. Flow will take place most readily through the larger vessels, since there is less friction in them. This can also be observed through the microscope by sectioning a branch, which has been transpiring in eosin solution, at the point of farthest advance of the eosin. At the same time flow is taking place more slowly in the smaller vessels.

Moreover, the smaller the vessel the greater the proportion of wet wall to maintain the sap under conditions of tension. Therefore, bubbles of gas will form in the larger vessels under excess transpirational pull before they will in the smaller. In a sufficiently small vessel, the influence of sorption may extend sufficiently near to the center of the vessel to prevent the formation of gas bubbles.

That these theoretical premises are correct is shown by the work of BODE (12), who observed, by careful dissection and direct observation, that air entered the largest vessels first. "Dagegen war das Eindringen von Luft in Gefäße mit einem Durchmesser unter  $10\ \mu$  nie zu Beobachten."

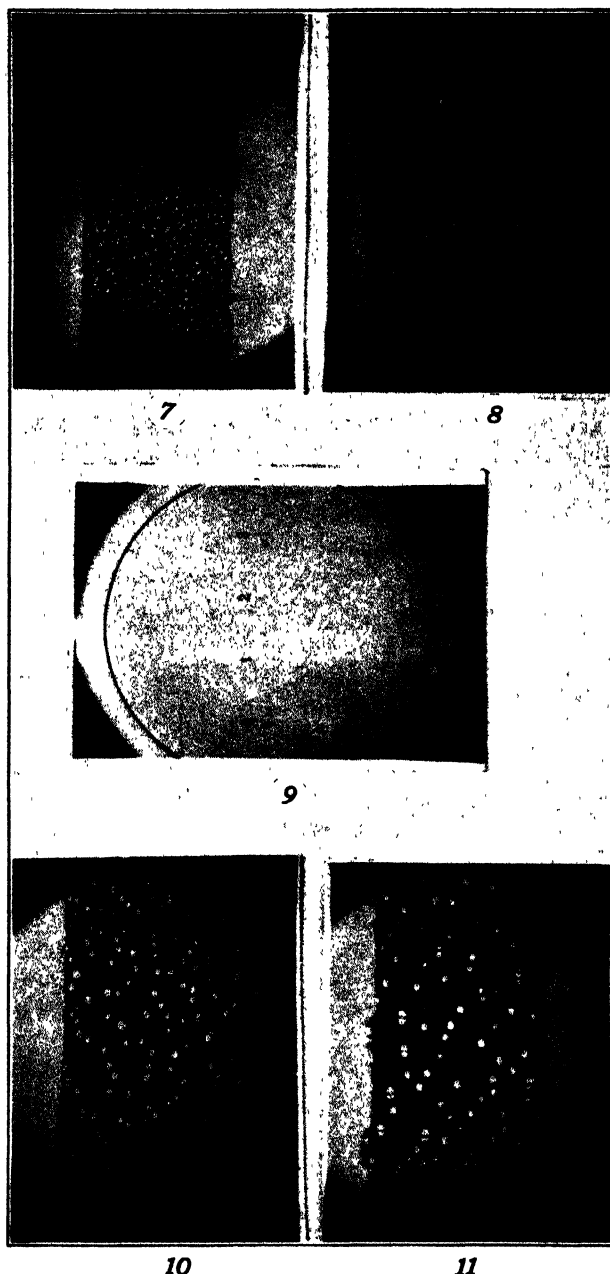
Through the courtesy of Dr. D. T. MACDOUGAL, I was able to examine the original slides prepared at the time that the above-mentioned studies on zonation were made. Photomicrographs of typical annual rings are presented in figures 7 to 11, together with a photomicrograph of a stage micrometer to the same scale (fig. 9).

The exactness with which the hypothesis offered fits these cases is further evidence in its favor.

In the pine (no illustration), where there are only tracheids and no tracheae, all of the elements are fairly uniform in diameter, with cross walls at frequent intervals; therefore there is conduction in all of them.

In the willow (fig. 7), each annual ring immediately starts off in the spring with large vessels, which continue until late summer when the size falls off rapidly and they become more numerous. The line of demarcation is sharper here than in the other two cases given later. According to my hypothesis, under conditions of stress, flow should take place only in the last millimeter (or less) of the annual ring. MACDOUGAL *et al.* report that "under midsummer conditions conduction is only in the later summer wood. Irrespective of the width of the annual ring, the width of the colored zone in the late summer wood is less than a millimeter."

In the alder (fig. 8), there are numerous and comparatively small vessels formed in the spring, becoming more numerous and rounded out as the



FIGS. 7-11.—Fig. 7, photomicrograph of transverse section of an annual ring from the xylem of willow. Fig. 8, same of alder. Figs. 10, 11, same of walnut. Fig. 9, photomicrograph of stage micrometer at same magnification as figures 7 to 11.

season advances, being very few in late summer; therefore the sap flows through the spring wood under midsummer conditions; my hypothesis again correlating with the observed fact.

In the walnut (figs. 10, 11), it was found that "the dye moves through the outer portion of the late summer wood and the innermost portion of early spring wood (of untylosed annual rings), leaving an intermediate zone through which there is no conduction. The zones of coloration are not as clearly defined as in the willow and alder." Applying my hypothesis, the reason for this is apparent on looking at the relative sizes of the vessels throughout the ring, small ones being formed on both edges with less distinct lines of demarcation than in the other two cases. Two rings chosen at random were photographed in this case to show the general applicability of the theory.

This hypothesis can be applied in ecological fields. For example, those plants with large ducts adapted to transporting water under moist conditions with mild transpiration, such as *Echinocystis*, succumb early under adverse conditions, compared with those plants having smaller vessels. The size of the ducts, to a large extent, is a hereditary factor. I have observed *Montia* growing in very dry and in very moist habitats, both of which had mature vessels 25  $\mu$  in diameter, the difference being in the number of such vessels. The hypothesis that the transpiration stream flows in the largest vessels in which an imbibed tensile fluid can be maintained under the conditions of the moment should prove to be a valuable addition to sap hydraulics.

### Summary

1. The transpiration stream moves through a system of xylem vessels, the walls of which are wet by the water moving through them. The xylem is surrounded on all sides and terminally by living cells. Flow through the xylem is a problem in hydraulics. The DIXON-ASKENASY tensile column theory is inadequate. Combined with the SACHS-LANGMUIR ideas on imbibition, a satisfactory hypothesis is obtained under which the walls of the vessels imbibe and support the weight of the water moving through them by tensile stress.

2. The corollary is offered that flow takes place through the largest vessels in which an imbibed tensile fluid can be maintained under the conditions of the moment. This can explain the observed zonation of conducting areas in the xylem, since the latter have vessels of varying diameters in definite seasonal zones.

3. The products of photosynthesis are transported through the phloem in solution. This solution is dehydrated when the nutrient materials are incorporated into growing cells, the excess water escaping by the path of

least resistance. A consideration of the morphological relations of phloem, meristematic tissue, and xylem, together with experimental evidence from guttation, shows that this phenomenon can account for root pressure and serve to repair broken water columns in the xylem.

4. In the absence of root pressure, the entry and movement of the transpiration stream can best be explained through tensile forces, arising by insolation in the mesophyll cells of the leaves, and transmitted through the forces of imbibition, osmosis, and cohesion. Evaporation against the imbibition pressure of protoplasm may be the determining factor in the activation of sap hydraulics.

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# EFFECT OF SOIL TYPE AND AERATION UPON ROOT SYSTEMS OF CERTAIN AQUATIC PLANTS

B. ELIZABETH DEAN

(WITH SIX FIGURES)

## Introduction

It is well known that the amount and movement of soil air are important factors in plant development. Because of the fact that many crop and aquatic plants are often forced to develop under conditions of minimal soil aeration, many investigations have been undertaken to determine the effects of restricted soil oxygen availability (1, 5, 7, 8, 14, 19, 20, 24). Such studies have disclosed that aeration is important in root respiration (8, 12, 20), nutrient absorption (5, 6, 18, 20), rate of elongation (2, 6, 7, 8, 13, 21, 24), development of hair roots (11, 23, 24), aerenchyma formation (3, 16, 21), adventitious root development (10, 24, 25), as well as in modifications of the gross anatomy of the entire root system (6, 8, 9, 24). The many papers reviewed by CLEMENTS (8) indicate that the rôle of oxygen in root activity is a primary factor influencing, and in wet soils controlling, the functions of the root system that may determine the destiny of the plant. But few studies (2, 4, 17) have been made of root response in greatly increased soil aeration, although some data are available on the effects of culture solution aeration (6, 13, 18).

This report embodies results of an experiment to determine the effects of soil aeration, increased well above the maximum usually encountered in nature, on several species of plants with relatively low oxygen requirements.

## Materials and methods

Four species of aquatic seed plants, *Hibiscus militaris*, *Acorus calamus*, *Sagittaria latifolia*, and *Typha latifolia*, were selected for use in the investigation. In the selection of all material of a species, as nearly uniform specimens as possible were obtained. All of the plants were placed in tanks in 10 inches of soil, with a 6-inch layer of tap water above the soil surface.

Growth was observed in three types of aerated and unaerated soils: clean coarse sand, highly colloidal clay, and muck. The aeration equipment consisted of perforated, coiled brass pipes at the bottom of each tank, fed through a pipe attached to the air-line with rubber tubing. Frequent titration and H-ion tests were made to check the alkalinity of the cultures. Slight alkalinity was partially maintained by the use of weak alkaline tap water to replace the water evaporated from the tanks. The investigation

was conducted in an experimental greenhouse with light, humidity, and temperature maintained as uniformly as possible. Measurements and photographs were made for comparative study of the entire root systems, together with details of the characteristic roots removed from the plants.

### Data and discussion

The results of the root development are given separately for each species, and are followed by observations correlating the root and top growth. Tables I-IV record the data of the investigation, and show the relative degree of root and top development of the four plants in each type of soil.

During the 15 weeks of growth period and at the time the plants were removed and critically studied, *Hibiscus militaris* showed several pronounced modifications. The plant in unaerated sand gave early evidence of shallow root development, for as soon as the above-ground shoot reached a height of a few feet it became top-heavy. After the water was siphoned from the cultures, the roots were found growing on and just under the surface of all unaerated soils. The plants were removed, and without exception the roots in soils of low oxygen content developed a shallow horizontal growth in striking contrast to the vertical growth of the roots through the aerated soils. Small, dwarfed root systems were pronounced in unaerated clay and muck (fig. 1, A, B). The aerotropic response of root growth to the source of air became evident when air was introduced from the bottom of the soils (fig. 1, C, D).

The development of heavy clumps of fibrous roots was found in aerated clay and muck. This clumping of roots, not found in sand, was probably influenced by soil nutrition. The root primordia were from the old root stalk in all soils, although adventitious roots developed 10-15 cm. above the root stalk on the plants from aerated substrata. The origin of roots from the old root stalk was supplemented by the development of clumps of fibrous members from the cut ends of old woody roots in all aerated soils and in unaerated sand.

It is apparent from the data (table I) that roots were modified by aeration. Some of the roots became decidedly woody in aerated cultures, but all remained succulent and flexible under unaerated conditions. A comparison of results also showed greater elongation of roots in all aerated cultures than in any unaerated soil. The rate of root and shoot elongation has been increased by aerating the substratum by ALLISON and SHIVE (2), HALL, BRENCHELEY, and UNDERWOOD (13), and HUNTER and RICH (17). Retarded growth and injury in higher plants, according to LIVINGSTON and FREE (20), may be attributed to decreased absorbing power of the root as a result of deficient soil oxygen. There was likewise more pronounced elongation of roots in aerated clay and muck cultures than in aerated sand.

**TABLE I**  
**ROOT AND TOP DEVELOPMENT IN *Hibiscus militaris* IN DIFFERENT SOIL CULTURES**

PLANT DEVELOPMENT	ROOT DIMENSIONS					
	SAND		CLAY		MUCK	
	UNAERATED	AERATED	UNAERATED	AERATED	UNAERATED	AERATED
	cm.	cm.	cm.	cm.	cm.	cm.
Average length of woody roots	0.0	30-35	0.0	50-55	0.0	52-58
Maximum length of woody roots	0.0	42.0	0.0	60.0	0.0	65.0
Average diameter of woody roots	0.0	0.3	0.0	0.5	0.0	0.5
Average length of succulent roots	10-15	20-25	5-10	25-30	2-6	25-30
Maximum length of succulent roots	25.0	30.0	13.0	35.0	10.0	35.0
Average length of primary laterals	1-4	4-8	2-5	5-10	1-3	7-12
Maximum length of primary laterals	10.0	12.0	10.0	15.0	5.0	15.0
Average length of secondary laterals	0.0	0.2-0.5	0.1-0.2	0.2-1.0	0.1-0.2	0.5-1.0
Maximum length of secondary laterals	0.0	1.0	0.5	1.6	0.2	2.0
	Tops					
Number of shoots per plant	1	2	3	5	2	6
Weight of tops in grams	120	155	78	370	89	465

Roots in all soils branched, but though secondary laterals were common in roots of all aerated soils, they occurred only occasionally on the surface roots of unaerated clay and muck, and never on the plants in unaerated sand. Twenty singly branched laterals per centimeter was the maximum amount of branching observed in roots in unaerated sand. Frequent branching was confined to the proximal portion of these roots (fig. 2, A), and branches became progressively shorter, as well as more irregular in length and distribution, toward the tip. Roots in the sand near the aeration coils showed more frequent branching (30 per cm.) of primary laterals near the tip. These primary laterals formed 5-20 short secondary branches at their

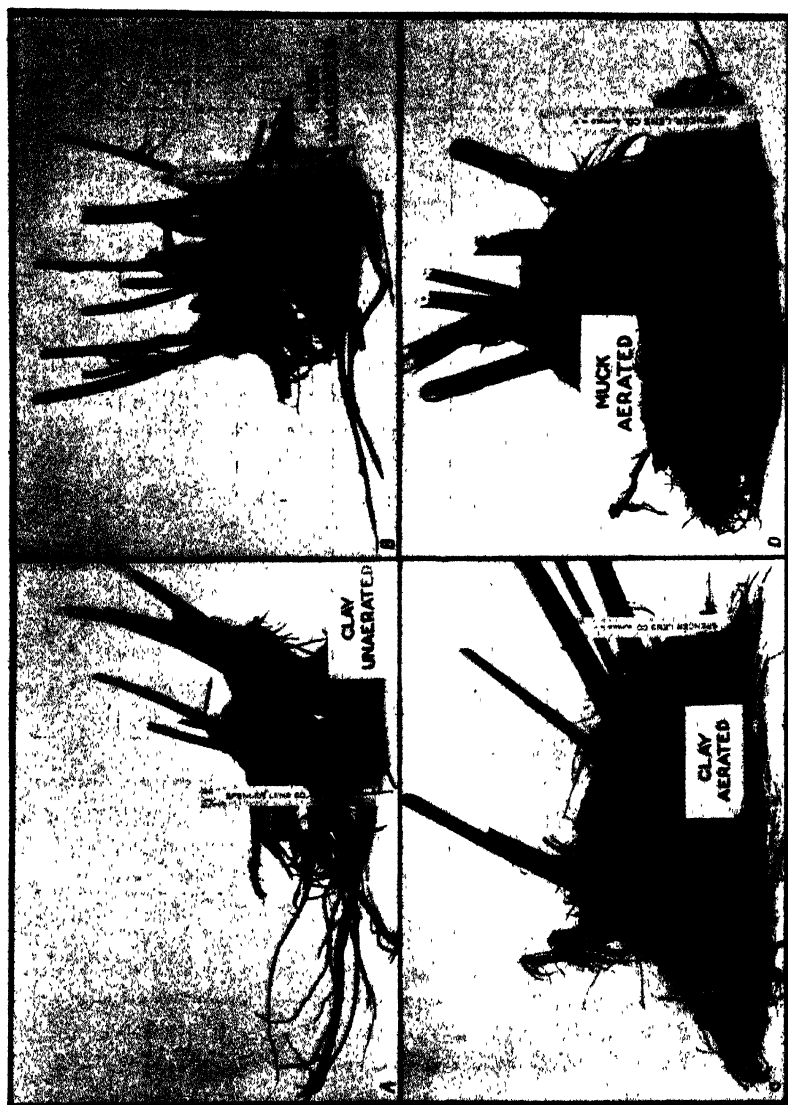


FIG. 1. *Hibiscus militaris* root system: A, unaerated clay; stunted root system with short roots developing only from root stalk of original planting. B, unaerated muck; meager root system with short fibrous roots developing from upper part of old root stalk. C, aerated clay; new woody and fibrous roots ramified throughout entire soil culture and about aeration coils. D, aerated muck; extensive system of new woody roots and heavy clumps of fibrous roots; large clump of roots in foreground.

proximal ends (fig. 2, *D*), another character not found on roots from unaerated sand.

Branching of laterals may be observed in roots of both the unaerated and aerated clay and muck cultures (fig. 2, *B, E, C, F*). These root details show that lateral branches are longer and more numerous on the roots receiving additional aeration (fig. 2, *D, E, F*). The irregular branching of laterals of first and second rank on roots from unaerated cultures is also in striking contrast to the long, evenly distributed laterals of respective rank on the roots taken from a clump near the air-lines. Lateral branching is restricted to the upper proximal end of roots from unaerated soils, but roots supplied with aeration have laterals of first and second rank practically the whole length of the roots.

Surface roots in unaerated clay and sand did not possess root hairs on branches that projected into the water, but they were found sparingly on the same type of roots in the muck cultures. Root hairs were observed on all roots of aerated cultures, but most abundantly on roots from the muck and clay. The most outstanding effects of aeration on *Hibiscus militaris*, particularly in compact clay and muck soils, were the marked increase in depth and extent of root growth, with the formation of secondary laterals in all soils.

The root systems of *Typha latifolia* in the different soils and aeration were examined after 56 days' growth, and again 44 days following replanting. All of the types of roots reported by WEAVER and HIMMEL (24) and by ROERDON (21) in their experiments with *Typha latifolia* were observed. These included: (1) branched water roots found abundantly in unaerated soils that developed at the highest point of origin on the basal node and grew upward and outward into the water to branch on or above the soil surface; (2) branched surface soil roots found in all unaerated soils and aerated sand; (3) deep vertically growing branched soil roots characteristic of all aerated soils; (4) unbranched soil roots found in all cultures. The results following alteration of soil or air factors illustrate clearly the facility with which this plant adjusts its type of root development to its physical environment.

The data (table II) show that branched water roots are found only in the unaerated cultures, and are noticeably longer and more branched in the muck soil than in either of the other soils. The water roots originated at the highest point of the basal node and grew up and out upon the soil surface. Approximately one-third of the roots on the plants in the unaerated cultures were water roots. These roots correspond to the superficial adventitious roots observed by EMERSON (10) on *Alnus* and other land plants growing in swamp land, and by WEAVER and HIMMEL (24) on *Typha latifolia* in their water-saturated cultures.

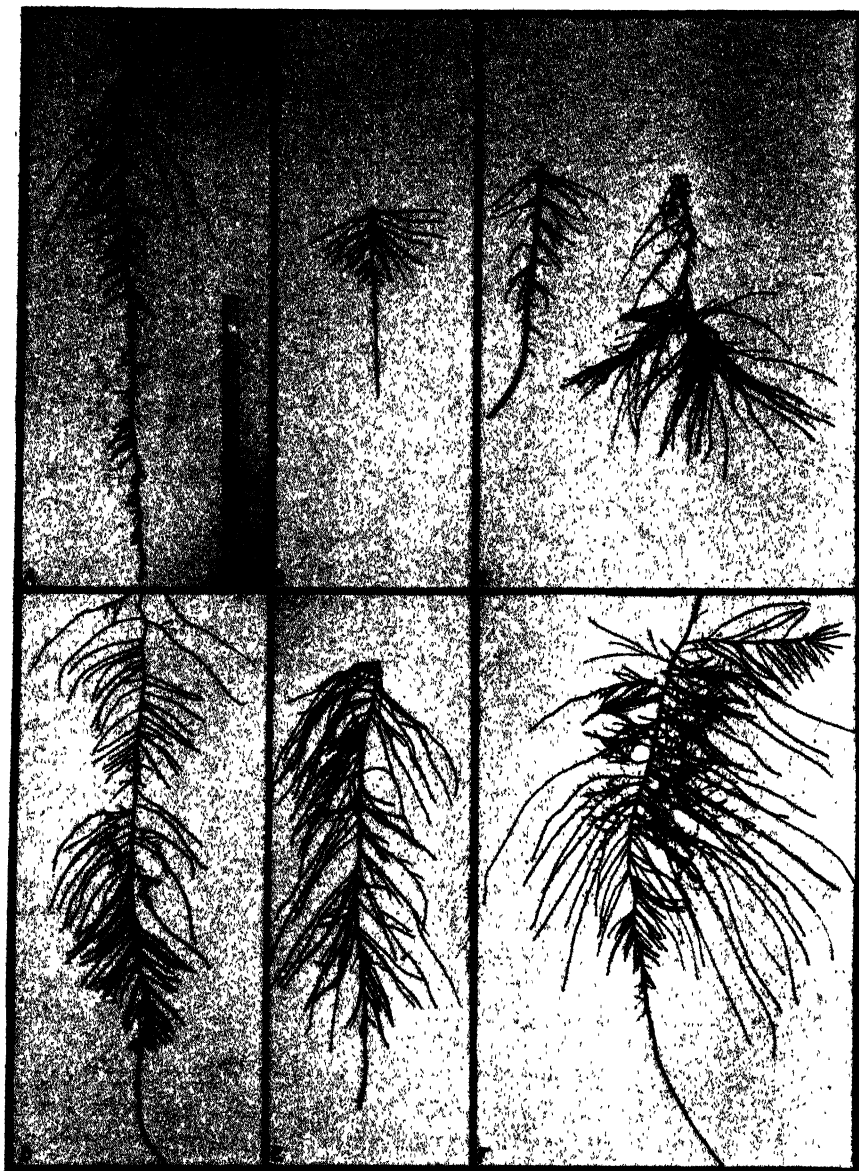


FIG. 2. Details of *Hibiscus militaris* soil roots: *A*, unaerated sand; typical root with primary laterals formed most frequently at proximal portion of root. *B*, unaerated clay; root showing a few short secondary laterals. *C*, unaerated muck; short scattered secondary laterals appearing at proximal region of roots. *D*, aerated sand; primary laterals showing secondary branching. *E*, aerated clay; typical root with secondary branching taken from near aeration coil. *F*, aerated muck; root with long primary and secondary laterals taken from clump of roots in region of aeration coil.

**TABLE II**  
**ROOT AND TOP DEVELOPMENT IN *Typha latifolia* IN DIFFERENT SOIL CULTURES**

PLANT DEVELOPMENT	ROOT DIMENSIONS					
	SAND		CLAY		MUCK	
	UNAERATED	AERATED	UNAERATED	AERATED	UNAERATED	AERATED
	cm.	cm.	cm.	cm.	cm.	cm.
A. Branched water roots						
Average length . . .	12-18	0.0	15-20	0.0	20-25	0.0
Maximum length . .	22.0	0.0	25.0	0.0	32.0	0.0
Average diameter of roots . . . . .	0.08	0.0	0.1	0.0	0.1	0.0
Average length of primary laterals	2.0	0.0	1.5	0.0	2.5	0.0
B. Branched soil roots						
Average length . . .	15-20	28-35	15-20	35-40	20-25	40-45
Maximum length . .	30.0	46.0	28.0	50.5	30.0	54.0
Average diameter of roots . . . . .	0.1	0.15	0.15	0.2	0.15	0.25
Average length of primary laterals	1-2	4-5	0.5-1	4-5	0.8-1.2	5-7
Maximum length of primary later- als . . . . .	2.5	6.0	1.0	7.5	1.8	10.0
Average length of secondary later- als . . . . .	0.0	0.2	0.0	0.2	0.0	0.3
C. Unbranched roots						
Average length . . .	10-15	20-25	20-25	35-40	35-40	40-45
Maximum length . .	24.0	40.0	47.0	56.0	50.0	68.0
Average diameter of roots . . . . .	0.15	0.15	0.18	0.2	0.2	0.2
D. Rhizomes						
Average length . . .	20-25	25-30	30-35	50-60	65-70	80-85
Average diameter . .	1.0	1.0	1.0	1.2	1.2	1.5
	TOPS					
Average length of leaves in centimeters . . . .	90	100	100	125	110	156
Number of leaves in sheath . . . . .	5-7	5-7	5-9	7-10	7-12	9-12
New aerial shoots on a rhizome . . . . .	2	5	4	9	20	39

In unaerated sand, 20-30 laterals per cm. developed uniformly to near the root tip. The water roots from unaerated clay and muck had from 30-50 branches per centimeter, and table II shows that they were 5-10 cm. longer than water roots from the sand soil. The branching was most irregular but proceeded to near the tip on roots from the unaerated muck.

Un-aerated and branched roots of lower origin than the water roots grew into the upper 5–10 cm. of the un-aerated soils. Unbranched and branched roots in aerated soils grew downward to the region of air supply, with only occasional roots growing horizontally near the soil surface. The longest roots and the most profuse branching of laterals were in the region of the aerating pipes, while branching in un-aerated conditions was on the upper half of each root. Secondary laterals never occurred on branched roots of un-aerated soil, but were found on roots provided with air.

Branched and unbranched soil roots were consistently longer in the aerated soils. The branched roots in both sand cultures were slender, with wrinkled surfaces. Laterals were correspondingly longer and twice branched in aerated soils (fig. 3, *D, E, F*). Singly branched laterals of roots from un-aerated soils were confined to the upper half of the root (fig. 3, *A, B, C*) while the twice branched laterals of roots from aerated soils developed down to the region of root elongation. Several centimeters of the distal ends of roots from aerated cultures were broken off as the roots were removed from the aeration coils. The average of several counts showed that the frequency of primary laterals on the soil roots was 5–30 per cm. from un-aerated sand, and 20–40 per cm. from aerated sand. In un-aerated clay, single branches developed 20–30 per cm., and in aerated clay 50–60 per cm. The most frequent branching was found on roots from the muck soils, 45–60 per cm. in the un-aerated condition and 50–65 per cm. with improved aeration. Lateral branching of soil roots in the aerated muck soil of this experiment showed a considerable increase in frequency over the lateral branching of similar roots reported by WEAVER and HIMMEL in their best aerated moist culture.

The unbranched roots were in the minority in all un-aerated soils, but were about equal in number to the branched soil roots in aerated cultures. From the data given in (C) of table II, it is apparent that unbranched roots are more extensive and more abundant in the muck soils than in clay or sand.

Root hairs were found only occasionally on water roots in un-aerated muck. While they occurred on all soil roots, they were most numerous on roots from aerated muck and clay. Root-hair development was influenced by aeration, soil nutrition, and position of roots. The fact that they were practically lacking on surface-growing roots in un-aerated soils is in accordance with observations of WEAVER and HIMMEL. Their occurrence on water roots of *Typha latifolia* and surface-growing roots of other plants in aerated muck may be explained, according to FARR (11), by the fact that calcium was present in the muck soils. SNOW (23) also, found that an increase of available oxygen produced root-hair development on various plants.



FIG. 3. Details of *Typha latifolia* soil roots. *A*, unaerated sand: (a) unbranched root, (b) branched root with single laterals on upper half. *B*, unaerated clay: (a) unbranched root, (b) branched root with single laterals confined to proximal region. *C*, unaerated muck; (a) unbranched root, (b) branched root with single laterals. *D*, aerated sand: (a) kinky unbranched root, (b) branched root with occasional laterals of second rank. *E*, aerated clay: (a) unbranched root, (b) branched root with secondary laterals. Distal end broken in removing from aeration coil. *F*, aerated muck: (a) unbranched root, (b) branched root with secondary laterals. Root tip broken off.

According to these investigations (11, 23), the presence of calcium and abundant oxygen, together with better nutrition, explain the fact that root hairs were most abundant in aerated muck soil, second in clay, and least in sand.

In the unaerated cultures new rhizomes were fewer than in the aerated soils. The development of rhizomes and the number of aerial shoots on plants grown in aerated muck exceeded those in all other cultures. One aerated specimen of *Typha latifolia* developed 12 rhizomes and 39 aerial shoots, with two rhizomes 125 cm. long.

Root development of *Typha latifolia* varied with the degree of aeration and the type of soil. These variations occurred in the mode of branching, the general habit, and the extent of development. In oxygen-deficient soil, branched roots developed above the soil surface, and with abundant oxygen all roots grew into the soil and downward to the source of aeration. Root development was greater in muck soils than in clay and sand, although the growth in all soils was greatly increased with aeration.

Specimens of *Acorus calamus* were removed from the cultures for study of their root systems 70 days after planting. Following the examination, they were replanted and taken up again at the end of 40 days. The root systems were made up of branched and unbranched roots, the former occurring in greater number. A comparative study of the development of these two types of roots showed a greater number of branched roots in aerated sand but a greater number of unbranched roots in aerated muck and clay. The branched roots were strong though very fine and hairlike.

Variations in growth with respect to the soil were more comparable with those found in *Hibiscus* than with those found in *Typha*, as differentiation of branched roots was not pronounced. The root systems in the unaerated soils (fig. 4, A, B) were shallow, with many branching roots forming tangled mats, while those in aerated soils (fig. 4, D, E) grew downward toward the air supply. Branched and unbranched roots as well as laterals were, without exception, longer in the aerated cultures. Neither the soil type nor aeration had any influence on the development of second laterals, as the branching habit in all cultures remained single.

A comparison of the details of roots (fig. 5, A-F) shows that lateral branching was less extensive on the roots from unaerated soils than on the deep vertical-growing roots from aerated soils. Lateral branching was outstanding on roots from aerated sand (fig. 5, D), and the laterals were 5-10 cm. longer than in roots from unaerated sand. Measurements (table III) show that laterals were fewer and 2-3 cm. shorter on roots from unaerated muck than from unaerated clay. Improved aeration produced a marked effect upon the growth of laterals in these two soils, the length being four times greater in aerated than in unaerated muck.

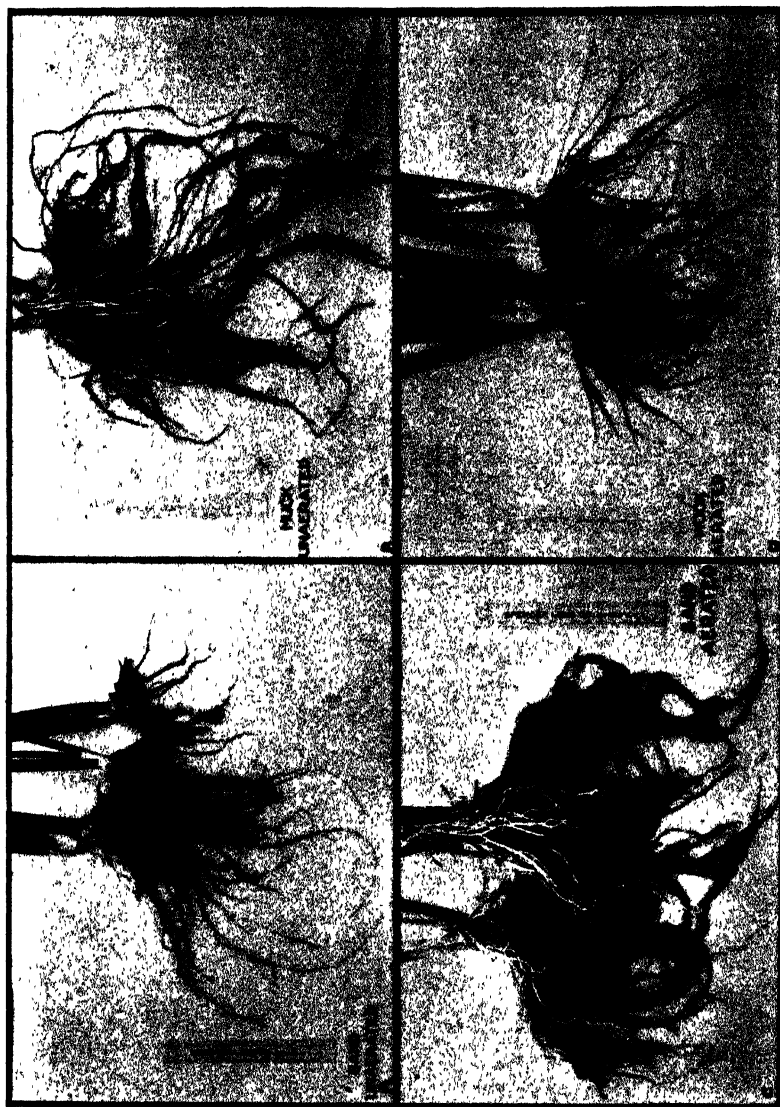


FIG. 4. *Acorus calamus* root system: A, un-aerated sand; wrinkled roots with short laterals growing horizontally under sand. B, un-aerated muck; roots growing in horizontal position on surface and upper regions of muck. C, aerated sand; roots showing kinky configuration from growing into sand. D, aerated muck; many roots broken and lost on removal from aeration coils.

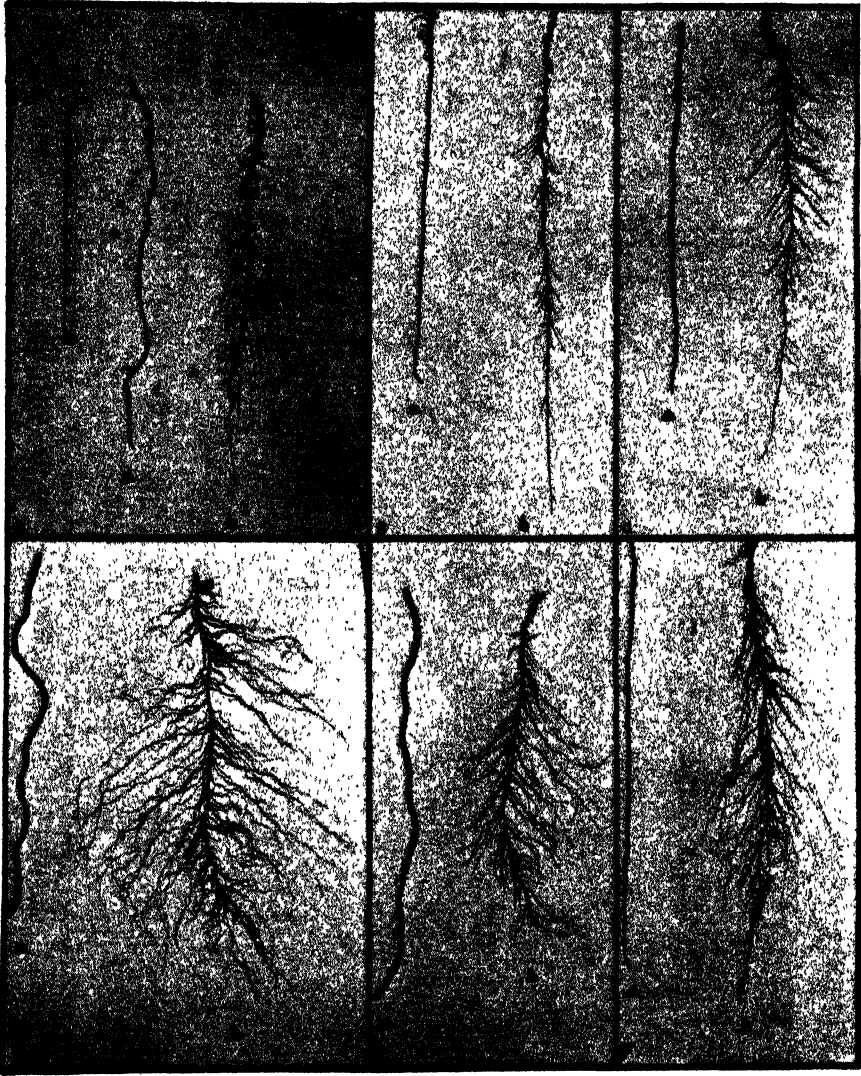


FIG. 5. Details of *Acorus calamus* soil roots. *A*, unaerated sand: (a) unbranched root, (b) typical branched root from same soil. *B*, unaerated clay: (a) practically unbranched root, (b) branched root with scattered laterals. *C*, unaerated muck: (a) unbranched root, (b) branched root from same culture showing shorter branching toward distal end. *D*, aerated sand: (a) unbranched root, (b) branched root from same plant with long delicate laterals developing down to root tip. *E*, aerated clay: (a) unbranched root, (b) branched root with lateral branching to tip. *F*, aerated muck: (a) unbranched root, (b) branched root with laterals developing to zone of elongation.

TABLE III

ROOT AND TOP DEVELOPMENT IN *Acorus calamus* IN DIFFERENT SOIL CULTURES

PLANT DEVELOPMENT	ROOT DIMENSIONS					
	SAND		CLAY		MUCK	
	UNAERATED	AERATED	UNAERATED	AERATED	UNAERATED	AERATED
	cm.	cm.	cm.	cm.	cm.	cm.
A. Branched root system						
Average length	13-20	23-30	18-22	30-35	20-25	30-35
Maximum length	25.0	35.0	30.0	40.0	30.0	40.0
Average diameter at proximal end	0.12	0.15	0.12	0.15	0.13	0.12
Average length	1-2	8-12	1-2	5-7	0.5-1.5	7-10
Maximum length of primary laterals	6.0	15.0	6.0	10.0	3.0	12.0
B. Unbranched root system						
Average length	7-12	15-20	18-22	20-25	20-25	25-30
Maximum length	20.0	30.0	32.0	40.0	35.0	40.0
Average diameter	0.12	0.15	0.15	0.2	0.2	0.18
	Tops					
Average height in centimeters	400	450	420	500	450	500

Root hairs were not observed on the surface roots of *Acorus calamus* in any of the oxygen-deficient soils, but occurred on the roots from aerated muck and clay.

The root habits just described show the same aerotropic response as *Hibiscus militaris* and *Typha latifolia*. The influence of the soil or aeration, however, was not observed to induce an increase of additional ranks of laterals as was found to occur with the *Hibiscus* and *Typha*.

*Sagittaria latifolia* plants were examined 44 days after planting. Branched roots developed at the base of the leaf stalks around the crown of the old rhizome which was used in the original planting. Only the very young roots were unbranched, a root response not following in the other species. All roots were singly branched and intricately tangled. In no case was lateral spreading of roots found on the soil surface, but all unaerated systems presented shallow horizontal growth away from the points of origin. Their branches were irregular and extremely stunted in comparison with those where aeration was supplied (fig. 6, A, B). The roots in the aerated soils grew to the air-lines, where they formed an interlaced

mesh of laterals throughout their extent of growth, and were frequently broken when removed from the aerating system.

Much bulkier root systems developed in unaerated sand than in clay and muck cultures with similar aeration. *Hibiscus militaris* showed like response to the same soil conditions. In contrast, *Typha latifolia* and *Acorus calamus* produced greatest growth in muck soil without aeration. *Sagittaria* likewise developed the greatest number of, and the most uniformly branched, roots in the sand of the aerated cultures. Although no nutrients other than those in the tap water were added to the sand, these cultures rather than clay and muck favored the growth of *Sagittaria*.

TABLE IV

ROOT AND TOP DEVELOPMENT IN *Sagittaria latifolia* IN DIFFERENT SOIL CULTURES

PLANT DEVELOPMENT	ROOT DIMENSIONS					
	SAND		CLAY		MUCK	
	UNAERATED	AERATED	UNAERATED	AERATED	UNAERATED	AERATED
	cm.	cm.	cm.	cm.	cm.	cm.
Average length of roots	5-10	30-35	4-8	20-25	3-5	22-25
Maximum length of roots .. .. .	15.0	42.0	12.0	35.0	10.0	35.0
Average length of primary laterals	0.5-1	3-5	0.2-0.5	1-3	0.5-1	1-5
Maximum length of primary laterals	1.5	6.0	1.5	5.0	1.5	2.0
	TOPS					
Number of leaves per plant.....	5	5	5	5	4	5
Average length of petiole .. .. .	42	45	32	56	30	39
Average length of blade .. .. .	13	16	13	23	9	15
Average width of blade .. .. .	7	10	7	17	6	9

Root elongation was increased approximately three times in this species by improving soil oxygen content of all soils (table IV). Length of laterals was increased four to six times in the aerated sand and clay. The irregularity of branching on the upper half of roots developed in insufficient soil oxygen (fig. 6, *C a*, *D a*, *E a*) is in contrast to the regular branching extending down to the region of elongation on aerated roots (fig. 6, *C b*, *D b*, *E b*).

Root hairs were found on roots of the *Sagittaria* plants in all cultures, but were more numerous on roots from the sand cultures than had been observed on the other species in similar cultures.

The species selected for this investigation are all hydrophiles adapted to life in poorly aerated soils. Their marsh bog habitats usually comprise a composite soil rich in organic matter. Experimental results (12, 15, 22) make it practically certain that injury from the toxic effect of carbon dioxide is more frequent than commonly supposed in all oxygen-deficient soils. From analysis of soil atmosphere, RUSSELL and APPLEYARD (22) found that rapid nitrification in organic soils was followed by an increase in carbon dioxide content and a falling off in the amount of oxygen. The percentage of carbon dioxide became extremely high and that of oxygen low in the water-logged soils. The muck cultures comparable with bog soil would have a high carbon dioxide content from decomposition of the organic matter in addition to that resulting from root respiration.

Varied types of soil were used to determine whether aeration produced uniform effect throughout the investigation. It has been pointed out in the results that root habits of all species varied by altering aeration and soil nutrition. *Typha latifolia* (table II) displayed the greatest adaptation to growth in stagnant soils by the development of finely branched water roots. This is evidently a response for securing oxygen peculiar to this plant, as water roots were rarely observed in aerated cultures. The occurrence of water roots probably accounts for the fact that *T. latifolia* plants grew in unaerated clay and muck. Although not so rank as in the corresponding aerated soils, they were by no means so dwarfed as the plants of *Hibiscus militaris* and *Sagittaria latifolia* from unaerated cultures which did not develop water roots.

In considering top development of the plants, it was observed that leaves or aerial shoots appeared from 7 to 12 days earlier in all species planted in the aerated soils. Color differences were not noticed in the foliage of *Sagittaria latifolia* and *Acorus calamus*, but the leaves of *Typha latifolia* and *Hibiscus militaris* grown in the sand cultures and unaerated clay were from the first a lighter green than the same species in the other cultures.

The data (tables I, III, IV) relating to the growth of plants above the soil indicate more abundant top development in all aerated soils for *Hibiscus militaris*, *Acorus calamus*, and *Sagittaria latifolia*. *Typha latifolia*, however, had greater growth in unaerated muck than in aerated clay and sand. The most rank top growth for *H. militaris* and *T. latifolia* was in aerated muck, but the tops of *A. calamus* and *S. latifolia* were greater in aerated sand than in aerated muck.

The correlation between root and top development is obvious regardless of the varied effects which were produced upon the species by the soils and

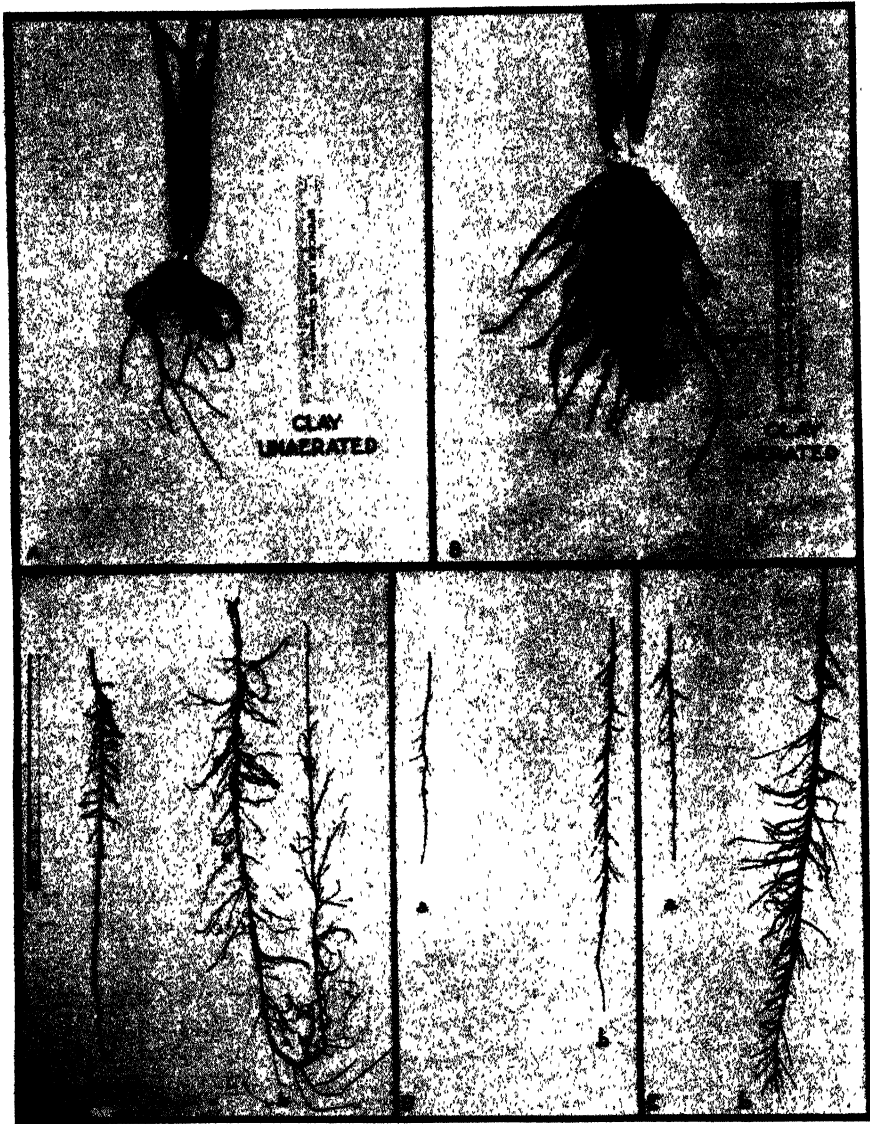


FIG. 6. *Sagittaria latifolia* root systems and root details. *A*, unaerated clay: entire root system stunted. *B*, aerated clay: root system shows effects of aeration. Root ends broken off in removal from aeration coils. *C*, sand cultures: (*a*) typical root from unaerated sand showing development of branches confined to upper half of root, (*b*) root from aerated culture with regular branching to region of elongation. *D*, clay cultures: (*a*) typical root from unaerated culture showing few laterals developed in comparison with (*b*), root from aerated clay showing more regular development of laterals to region of elongation. *E*, muck cultures: (*a*) root from unaerated culture, (*b*) root from soil receiving aeration.

aeration. With increased aeration, there followed uniformly greater top growth than occurred in corresponding plants in unaerated soils. HUNTER and RICH (17) found that the rate of transpiration and the intensity of respiration were increased in the shoots of *Impatiens balsamina* with soil aeration. Accompanying these observations there was an increase in surface area of both stem and leaves with increased functional activities.

*Hibiscus militaris* was the only species to mature. The facts that the plants in aerated cultures flowered normally and that all buds blasted and dropped from the plants in unaerated soils are in accordance with the findings of ALBERT and ARMSTRONG (1), who report an increased shedding of cotton fruit buds from plants associated with soil air containing high carbon dioxide and low oxygen percentages. ALLISON and SHIVE (2) likewise found that the average total yield of soy beans, when a maximum supply of oxygen was maintained, was greater than yielded from unaerated cultures.

The effects of increased aeration varied with the different species in the different soil types. The results indicate that root and shoot development was increased in all aerated cultures regardless of soil type, while the extent of the plant growth reached a maximum in aerated muck soil, with the exception of *Sagittaria latifolia* which grew best in aerated sand. The deviation from the general behavior, noted in *Sagittaria*, appears attributable to the fact that it possesses a high degree of vegetative activity and ability to develop adventitious roots for aeration. This plant seems to rely more upon its aqueous substratum than upon the soil for its nutrients, especially under conditions of poor aeration.

### Summary

1. Soil aeration, involving access to oxygen and removal of carbon dioxide, appears to play an important rôle in plant growth through its direct effect on root systems. Somewhat abundant soil aeration does not appear superoptimal, although it induces structural characteristics not found in plants under approximately normal soil conditions. Although the magnitude of these formative changes in plants varies with the soil type, the general tendency in the species studied was rather uniform.

2. *Hibiscus militaris*, *Typha latifolia*, *Acorus calamus*, and *Sagittaria latifolia* developed bulkier root systems and larger tops in aerated cultures.

3. Relative growth and development of all species were greatest in muck, second in clay, and least in sand with the following exceptions: *H. militaris* had a maximum growth in unaerated sand, and *S. latifolia* attained maximum growth in unaerated and aerated sand cultures.

4. The root systems of all species showed aerotropic response. In unaerated cultures the plants established shallow and surface root systems,

while with aeration the roots ramified the soil with the greatest growth in the region of the aerating coils.

5. Increased root elongation was accompanied by corresponding increment in root diameters in aerated soils, with this exception: root diameters of *Acorus calamus* were greater in unaerated muck than in aerated.

6. Laterals were longer and branched nearer the root tips in all aerated soils than in unaerated.

7. Second-rank laterals occurred in *H. militaris* roots in all cultures except unaerated sand, and on *T. latifolia* roots in all aerated soils but never in unaerated ones.

8. Roots of *S. latifolia* and *A. calamus* remained singly branched in all cultures.

9. Branched surface-growing roots were developed by *H. militaris*, *T. latifolia*, and *A. calamus* in all unaerated soils.

10. Root hairs did not occur on surface-growing roots in unaerated sand or clay, but were found in unaerated muck. They were much more numerous on roots from all aerated soils.

11. Pronounced formative changes may be induced by artificially increased soil aeration, and the effects of rapid renewal of the soil atmosphere are by no means so injurious to the plant as restricted soil aeration is apt to be.

This investigation was carried on at the University of Iowa, under the advice of Dr. W. F. LOEHWING, whose assistance is much appreciated.

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# INFLUENCE OF LEAF DESTRUCTION BY SULPHUR DIOXIDE AND BY CLIPPING ON YIELD OF ALFALFA<sup>1</sup>

GEO. R. HILL, JR. AND M. D. THOMAS

(WITH SIX FIGURES)

## Introduction

Exposure to a sufficiently high concentration of sulphur dioxide for a long enough period of time always injures the leaves of alfalfa. Almost invariably the fully grown, highly functional leaves show the injury first, and are more severely injured than the rest. If the concentration is high enough, for example, to produce markings within an hour, after possibly three-fourths of this time has elapsed the leaves become noticeably stiffened, as though the cells had greatly increased in turgidity. The turgidity seems to increase until certain areas in the leaf (those along the margins and between the veins, where the intercellular air spaces are most abundant) suddenly become flaccid and take on a water-soaked aspect, giving the leaf a mottled appearance. If the fumigation be continued, additional marginal or interveinal areas on these leaves already affected, and corresponding areas on hitherto unaffected leaves, become similarly flaccid.

With a few hours of sunshine after the fumigation, these flaccid areas bleach almost to an ivory color. The areas of each leaf which do not become flaccid remain green. Usually the green areas remaining after a heavy fumigation lie along the midrib and the principal veins of the leaf. They are separated sharply from the bleached areas.

The marked areas of an alfalfa leaflet after any fumigation may vary from one spot the size of a pin-point to several larger ones, which may involve 50 per cent. or more of the area of the leaflet. A given fumigation may destroy more than 50 per cent. of the green tissue of some leaflets and yet leave 50 per cent. of the leaves entirely unmarked. Basal leaves are not often marked, and leaves that have not attained their growth only rarely. In all of our experience with alfalfa, a marked stem has never been found. When a majority of the green tissue of a leaflet is destroyed by sulphur dioxide, the leaflet curls and frequently drops from the plant.

This type of injury is called "acute." Occasionally another type of sulphur dioxide injury on alfalfa leaves is encountered, a type that mani-

<sup>1</sup> Contribution from the Department of Agricultural Research of the American Smelting & Refining Company, Salt Lake City, Utah.

Grateful acknowledgment is made to MILTON R. BERNTSON, JOHN N. ABERSOLD, EVAN HARRIS, A. F. BARNEY, IVAN E. BURGOYNE, THOMAS BUNKALL, LYNN BROWN, and GEORGE GARDNER for their help in carrying forward this work.

fects itself several days after the fumigation. This is evidenced by a rather rapid disappearance of chlorophyll from parts of an otherwise apparently normal leaf, as if the chloroplasts in certain areas of the leaf, three or four days after the fumigation, had ceased to manufacture that unstable pigment. This type of injury is called "chlorotic" (chronic injury by STOKLASA 8).

What is the effect upon yield of a fumigation which destroys a portion of the leaf tissue? Does the green tissue that remains after the fumigation continue to function, and if so, at how nearly normal a rate? If there is a decrease in yield following a sulphur dioxide fumigation, is that decrease proportional to the leaf area destroyed?

In this paper the fumigation apparatus and the methods of growing, fumigation, measuring the injury, and harvesting the plants are described; also yield data as affected by sulphur dioxide lesions and by clipping are presented. The influence of various environmental and physiological factors on leaf destruction by sulphur dioxide, and upon the absorption of that gas by plants, will be considered in subsequent papers.

### Historical review

There are many papers, mostly foreign, on the relations of industrial wastes, particularly sulphur dioxide and sulphur trioxide, to agricultural production. The problem has long been recognized as one of considerable scientific and economic interest. The European investigations have been summarized by HASELHOFF and LINDAU (1) and by STOKLASA (8). In this country the principal report of the problem is by the Selby Smelter Commission (2), which also contains an extensive bibliography with abstracts of most of the earlier papers. VERPLANCKE (14) has discussed the subject in a general way; and in a review of the literature by JOHNSON (3), reference has also been made to recent investigations.

The earlier investigators gave descriptions of the lesions produced on many different plants, and called attention to the acute type and to the chronic, or chlorotic type of  $\text{SO}_2$  markings. They also suggested an order of magnitude of the exposures to sulphur dioxide which might cause injury to vegetation. The Selby Commission developed a technique for fumigating plants with sulphur dioxide similar to that subsequently improved upon by O'GARA, described later, and carried out an extensive study of the action of  $\text{SO}_2$  on barley. The Commission's report for the first time gave quantitative information as to the effect of concentration and duration of fumigation upon leaf destruction. They showed that "severe" lesions might reduce the yield of grain as much as 40 per cent., depending on the stage of growth when the fumigation occurred, and that it was somewhat proportional to the intensity of the fumigation. They found no reduction in yield

when the leaves were injured only slightly or not at all. Similarly JOHN-SON (3), working on the problem of invisible injury, found no measurable injury to wheat even after very prolonged exposure to sulphur dioxide but at concentrations too low to produce visible markings. So far as the writers are aware, there are no investigations in the literature on the relation of leaf destruction by sulphur dioxide to yield of alfalfa.

In 1914 the American Smelting & Refining Company established a Department of Agricultural Research to study the smelter smoke problem. This Department, under the leadership of the late Dr. P. J. O'GARA, carried out a considerable number of experiments during a period of nine years, the details of which, for the most part, remained unpublished in 1923, when O'GARA became ill and was unable to participate further in these researches. Some of the results of these studies, however, were published in a paper by WELLS (15), who discussed the conditions favoring the dispersion of sulphur dioxide from a smelter. SWAIN (9) and THUM (13) have summarized the smoke litigation in Salt Lake Valley, Utah, giving some details of O'GARA's work, including photographs of the apparatus and of the experimental plots in the field. O'GARA discussed some of his conclusions in 1917 (4), and also before the American Institute of Chemical Engineers (5). Only an abstract of the latter paper was published. Further, O'GARA circulated in a limited way a list which indicated the relative resistance of a number of plants to sulphur dioxide.

### Fumigation apparatus and technique

The plan of procedure developed by O'GARA in his closed-cabinet fumigation experiments has been largely followed in these studies. O'GARA used small field plots 5 feet square, suitably spaced in the field so that they were readily accessible. He fumigated these plots with various synthetic mixtures of sulphur dioxide and air, under a cabinet  $180 \times 180 \times 120$  cm., made of celluloid mounted on a wooden framework. The sulphur dioxide was delivered from a bottle through a needle valve to an empirically calibrated capillary flowmeter, which indicated the rate of flow of the gas. An adjustable water trap before the needle valve enabled the operator easily to obtain a controlled and very steady stream of gas, which was sent into a fan delivering about 10,000 liters of air per minute. The gas mixture was then conveyed through a 15-cm. pipe to the center of the top of the fumigation cabinet, where radial baffle plates distributed the stream uniformly downward to all parts of the cabinet. As the cabinet was not gas-tight and had no specific outlet, the displaced air-SO<sub>2</sub> mixture leaked out wherever it could. The concentration of SO<sub>2</sub> in the air in the cabinet was determined intermittently by the starch-iodine method of MARSTON and WELLS, as described by the Selby Commission (2). This method consisted

of drawing a definite volume of the  $\text{SO}_2$ -air mixture into a partially evacuated 20-liter bottle containing a starch-iodine solution of known strength, absorbing the  $\text{SO}_2$  by vigorous agitation, and titrating this solution with iodine or sodium thiosulphate to a standard light blue color. The amount of  $\text{SO}_2$  thus determined by analysis usually agreed well with the values calculated from the volumes of  $\text{SO}_2$  and air as indicated by the  $\text{SO}_2$  flowmeter and a pitot tube in the air line. Humidity readings were taken at intervals with the whirling psychrometer, and the condition of the plants was observed after the fumigation.

The writers planned to control the humidity and light in the fumigation cabinet, and to measure continuously the sulphur dioxide in the cabinet and also the amount of it absorbed by the plants. O'GARA's apparatus was therefore modified with these objectives in mind.

The fumigation cabinet was of similar type, consisting of a light steel framework  $195 \times 195 \times 150$  cm., covered with celluloid, and made nearly gas-tight. It was mounted on a galvanized iron base provided with a 20-cm. outlet pipe on one side, and a trough on top in which a water seal with the bottom of the cabinet could be made. It was found later that a more satisfactory seal could be made by using a pad of felt 2 cm. thick in the trough, instead of the water. The base was placed around the plot, made level, and its lower edge sealed with soil. When the cabinet was placed on this foundation, anemometers in the intake and outlet pipes of the system indicated that about 80-100 per cent. of the air which entered the cabinet went out by the outlet pipe.

The air was analyzed for sulphur dioxide continuously and automatically by means of a sulphur dioxide autometer, as described later. This machine sampled the air on a 2-minute schedule alternately from a point just below the delivery pipe in the top of the cabinet and from the outlet pipe. The unaccounted-for air was assumed to have escaped at the average concentration of intake and outlet gas. Sulphur dioxide was added to the system with an arrangement similar to that used by O'GARA and already described, except that the apparatus was inclosed and thermostated. The intake of the fan was provided with a shutter so that the volume of air delivered to the cabinet could be varied as desired.

The cabinet was wired so that lamps and reflectors could be mounted above the plants. When the cabinet was covered with canvas, definite light intensities could be maintained, depending on the number and size of the lamps employed. It has not yet been practicable, however, to attain light intensities approaching sunlight. For determining light intensity, the cabinet was provided with three thermocouple light meters. These thermopiles had sixteen junctions and were constructed of 5-cm. pieces of no. 24 nickel-chromium: constantan wire. They were mounted in a wooden box

about 12.8 cm. square and 2.5 cm. thick. One set of junctions was spread out in a row in a section of the box provided with a glass window, the junctions being painted black and the interior of the compartment being painted white. The cold junctions were placed in a dark but ventilated section of the box. This arrangement gave a reading of about 50 millivolts with full sunlight intensity. More recently, the new Weston "photronic" cell shunted across a resistance box has been used with excellent satisfaction. With the proper shunting resistance, the cell and thermopile gave nearly identical readings in the range from about 10 to 100 per cent. of full sunlight intensity. At lower light intensities the accuracy and sensitivity of the thermopile decreased, whereas the photronic cell remained useful, even in dense shade. The cell was also practically instantaneous in its action, and readily indicated differences resulting from the angle of incidence or clouds in the sky.

For making humidity determinations the cabinet was provided with wet and dry bulb thermometers. One pair of thermometers was mounted in the upper part of the cabinet and was provided with a small fan to cool the wick of the wet bulb. Another pair was mounted in the base near the outlet pipe where the movement of the air in the pipe furnished sufficient draft to cool the wet bulb. The relative humidity was maintained at any desired percentage by blowing an atomized spray of water into an intake pipe leading to the fan and also by introducing steam into the same intake pipe. The spray alone was found to be insufficient to maintain the relative humidity on dry summer days above 60 to 70 per cent.; but when this supply of moisture was augmented by steam, the humidity could be maintained between 95 and 100 per cent. Use of the water spray alone ordinarily causes the temperature of the cabinet to fall from 1° to 4° below the outside temperature. While such lowering of the temperature might have a slightly adverse influence on the experiment, it was also advantageous in preventing moisture from condensing on the walls of the cabinet. Silica gel was used to lower the relative humidity.

A continuous, automatic analytical method for determining sulphur dioxide in air was a fundamental requirement for this work. This was partly attained in 1927, when a machine was constructed (10) which automatically drew a measured volume of gas through a measured volume of standard starch-iodine solution at a uniform continuous rate and discharged the solution into a bottle every two minutes, ready for titrating the excess of iodine. In 1928 this apparatus was modified (11) so as to absorb the gas in a slightly acidulated solution of hydrogen peroxide, which oxidized the  $\text{SO}_2$  to sulphuric acid. The acid thus produced made the solution a better conductor of the electric current, and it was possible to follow the absorption with an electrical conductivity recorder, thus obtaining a con-

tinuous record of the concentration of the sulphur dioxide. The apparatus has been further modified (12), so that it is now well adapted to determine with great precision the absorption of the gas by plants in a fumigation cabinet at both low and high concentrations.

The alfalfa plants used were as uniform and comparable as it was possible to secure them. Twenty uniform vigorous seedlings of "common" alfalfa were selected for each plot, in which there were four rows with five plants in each row. The plants thus spaced, when mature, produced approximately fifty stems to the plant. When in the early blooming stage, these stems had approximately fifty leaves (150 leaflets) to the stem. The plots were irrigated by the flooding method often enough to keep the soil well supplied with moisture.

A considerable number of fumigations of alfalfa were carried out under diverse conditions, which produced leaf destruction to different extents. The percentage of leaf destruction was estimated for each plant, either by counting the leaves that were marked and measuring the marked area, or by comparing with a set of standard marked plants, the percentage of bleached area of which had been carefully measured. The estimates of the individual plants, made independently by different observers, were seldom found to differ by more than 10-15 per cent., and the average values for the plot were found to agree within 4 per cent.

At harvest each plant was cut and tied separately, and its green weight obtained immediately. The plants were then placed on end in the greenhouse until air-dry, and again weighed individually, samples being taken to determine the oven-dried weight of the material. A number of individual plants from various parts of the room were weighed at intervals during the time the other plants were being weighed, and were found to vary only to a negligible extent. Accordingly the air-dried weights of each group of plants were considered to be comparable among themselves.

In working out the yield data, the yield of a treated plot was compared with the yield of the same plot on preceding and succeeding untreated crops during the same year, thereby eliminating any influence of variation between the different plots. The yield of successive crops of alfalfa decreases rather regularly during the season (6, 7). Factors were, therefore, determined for each crop by means of untreated check plots scattered throughout the field. By multiplying the yield of each of the crops from each plot by its corresponding factor, the yields of the different crops were placed on a comparative basis. The factors for the 1929 yields were found to be as follows: first crop 1.00, second crop 1.46, third crop 2.00, fourth crop 2.75; and for 1930: first 1.00, second 1.51, third 1.61, and fourth 1.99. It was then possible to compare the yield for any treated plot with preceding or subsequent yields of the same plot when untreated. The yield of a treated

crop divided by the expected yield of that crop gave the percentage of yield as a result of the treatment. This method of calculation was complicated by the fact that, in case of the very severe treatments, the next crop subsequent to the treatment might show a reduction in yield also. In such case comparison was made on the basis of the preceding crop, or of the second subsequent crop. Not more than two of the four crops on any plot were treated during a season.

### Alfalfa yield data

A. O'GARA'S DATA.—O'GARA conducted many experiments in which he sought to determine the effect on yield of sulphur dioxide fumigations of varying degrees of severity. He recorded the extent of leaf destruction, but in qualitative terms only. It is therefore impossible to evaluate the experiments in which appreciable markings were observed, although they apparently agree in order of magnitude with our own. Accordingly this material is not considered further.

O'GARA also carried out two important experiments with the idea of determining the yield as influenced by repeated low concentrations which did not produce markings. He fumigated three pairs of plots simultaneously, with and without sulphur dioxide, 30 minutes daily for 27 days. On the subsequent crop he reversed the treatment on 42 days. He expressed the yield of the crops fumigated with  $\text{SO}_2$  as the average percentage of the yield of the check plots for both crops. This method tended to eliminate plot differences. Table I has been compiled from O'GARA's unpublished data. This table indicates that so long as fumigations do not produce more than traces of markings, there is no significant reduction in yield resulting from a considerable number of short fumigations.

B. YIELD OF ALFALFA AS AFFECTED BY ACUTE SULPHUR DIOXIDE MARKINGS.—During the season of 1929, a great number of plots were given single fumigation treatments on chosen crops, in which varying percentages of leaf tissue were destroyed. The results are shown in figure 1, in which the yield is plotted against the percentage of leaf area destroyed. Since the number of fumigated plots is so large, it is not practicable to present the individual yield data in a table. In figure 1 the various crops which were treated are shown by different kinds of points, as indicated in the legend. The equation of a straight line through these points, worked out by the method of least squares, is  $y = 98.6 - 0.265x$  . . . . . (1a) in which  $y$  is the yield of dry matter expressed as percentage of the check, and  $x$  is the percentage of the total leaf area destroyed. The number,  $n$ , of plots fumigated in this experiment was 80, and the standard deviation,  $S_y$ , of the individual plot yield from the line is 5.85 per cent. The somewhat high value of the coefficient of correlation,  $r = 0.715 \pm 0.055$ , indicates

TABLE I  
INFLUENCE OF REPEATED FUMIGATIONS WITH SMALL CONCENTRATIONS OF SULPHUR DIOXIDE ON YIELD OF ALFALFA; O'GARA'S DATA, 1915

DATE	CROP	NO. OF FUMIGATED PLOTS	NO. OF CHECK PLOTS	NO. OF FUMIGATIONS PER PLOT	DURATION OF FUMIGATION	CONCENTRATION OF SO <sub>2</sub>			YIELD DRY WEIGHT PER PLANT (PERCENTAGE OF CHECK)				LEAF DESTRUCTION
						MIN.	MAX.	AVE.	CROP	AVE. OF TWO CROPS	STAND. DEVIATION	STAND. DEVIATION OF MEAN	
8/3-9/4	2nd	3	3	27	min. 30	p.p.m. 0.3	p.p.m. 2.0	p.p.m. 0.91	100	%	%	%	None
9/8-10/3	3rd	3	3	41	30	0.4	2.3	0.62	100	100	12.0	5.0	None
8/3-9/4	2nd	3	3	27	30	0.9	4.1	1.82	83				Slight markings
9/8-10/30	3rd	3	3	42	30	0.7	3.2	1.21	107	95	20.0	8.0	Slight markings

that considerable confidence may be reposed in equation (1a) as expressing the relation between yield and leaf destruction for a single fumigation. The difference between the origin of this curve at 98.6 per cent. yield and at 100 per cent. is without significance, considering the large value of the standard deviation of the yield from the curve. The equation indicates that a fumigation which destroys all of the fully matured leaf tissue of a plant at any given time could be expected to reduce the yield about 28 per cent., and that the destruction of any given percentage of leaf tissue could be expected to reduce the yield by that same percentage of 28 per cent.

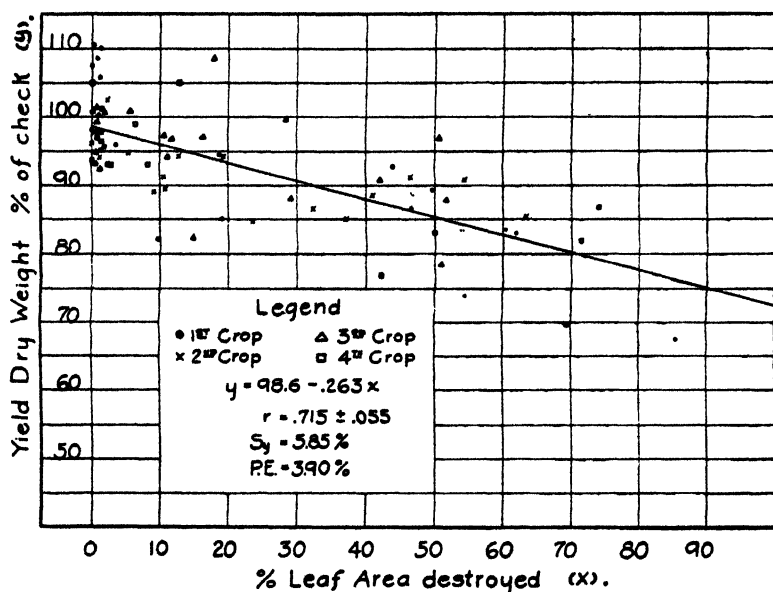


FIG. 1. Effect of the destruction of various percentages of leaf tissue by a single sulphur dioxide fumigation on the relative yield of alfalfa. Each point represents the yield of the fumigated crop from a plot as compared with the untreated crops from the same plot; 1929 data.

In 1930 the attempt was made to produce the same percentage of leaf destruction on certain crops in each of one, two, and three fumigations. The percentage of leaves which were marked was determined, as well as the percentage of the leaf area destroyed. In the multiple fumigations these values are expressed as the average of the individual percentages for each fumigation. These data are presented in figures 2 and 3.

In the upper chart of figure 2 are given the 1930 data. These duplicate the 1929 experiments recorded in figure 1, except that the points are segregated as to stage of growth at time of fumigation, instead of as to the crop fumigated. The equation of the best-fit straight line is:

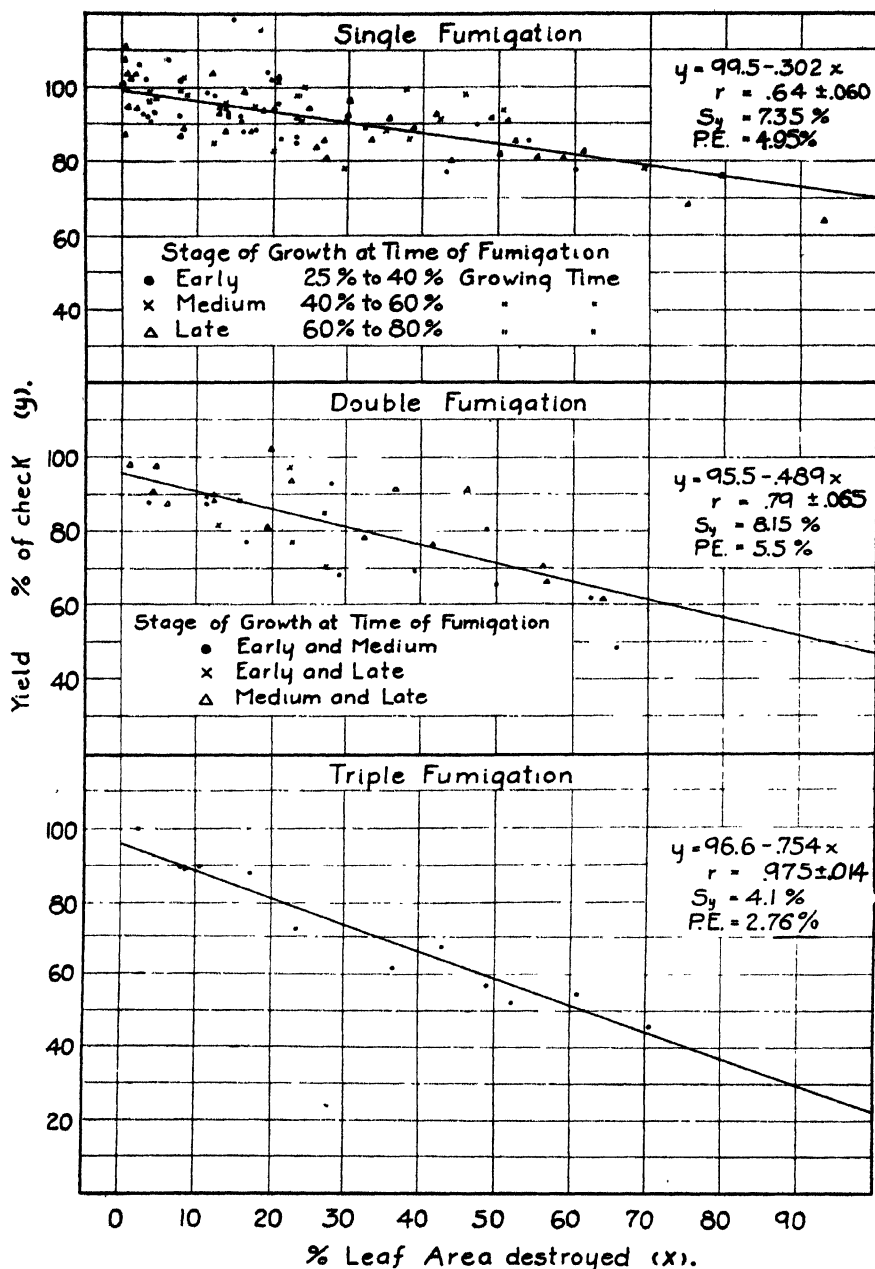


FIG. 2. Effect of the destruction of various percentages of leaf tissue by one, two, and three sulphur dioxide fumigations on a single crop on the yield of alfalfa. Yield of fumigated crop is expressed as percentage of yield of untreated crops on the same plot; 1930 data.

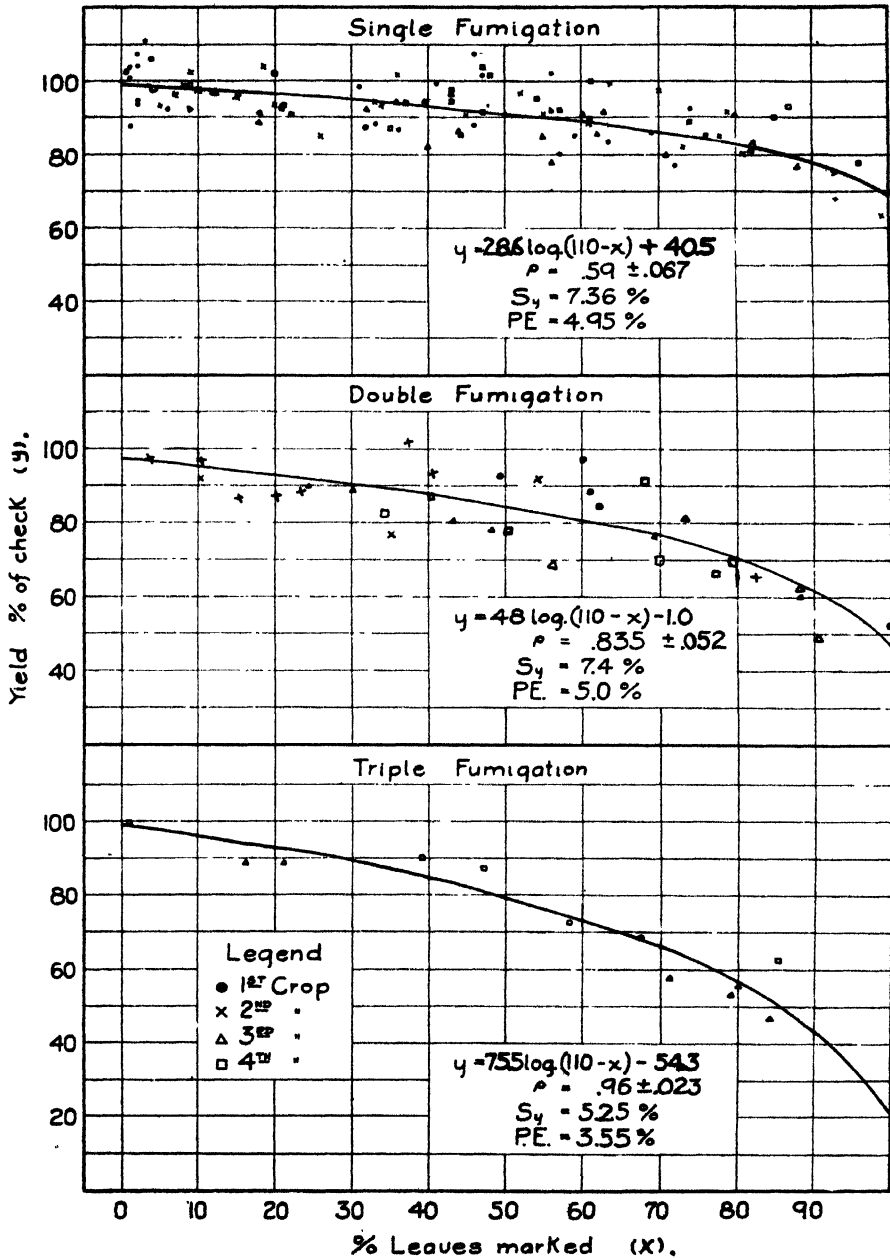


FIG. 3. Effect of the destruction of various percentages of leaf tissue by one, two, and three sulphur dioxide fumigations on a single crop on the yield of alfalfa. Leaf destruction is shown as percentage of the total number of leaves which were marked in any degree. Yield values are the same as in fig. 2; 1930 data.



For the double fumigation:

$$\begin{aligned} y &= 48.0 \log (110-x) - 1.0 & . & . & . & . & . & . & . & . & (5) \\ n &= 34 \\ S_y &= 7.4 \text{ per cent.} \\ r &= 0.835 \pm 0.052 \end{aligned}$$

For the triple fumigation:

$$\begin{aligned} y &= 75.5 \log (110-x) - 54.3 & . & . & . & . & . & . & . & . & (6) \\ n &= 12 \\ S_y &= 5.25 \text{ per cent.} \\ r &= 0.96 \pm 0.023 \end{aligned}$$

In figure 2 the point designations refer to early, medium, and late stages of growth respectively, and in figures 1 and 3 similar designations refer to first, second, third, and fourth crops.

These data indicate that any given percentage of leaf destruction of alfalfa is accompanied by approximately the same percentage of decrease in yield, regardless of the stage of growth or whether it is the first, second, third, or fourth crop, providing time is allowed for the treatment to take effect. Of course a very late fumigation, just before harvest, could hardly be expected to affect the yield; but in these experiments the crop was not harvested until at least seven to ten days after the last fumigation, thus allowing the influence of the fumigation to be fully reflected in the yield. Similarly the alfalfa had been growing a week before the experiments were begun.

The six curves shown in figures 2 and 3 are brought together in figure 4, in order to compare and contrast, in their effect on yield, the different percentages of leaf destruction and the percentages of leaves showing markings. A horizontal line, drawn from any point on any one of the dotted-line curves to the point that it intersects on the corresponding straight-line curve, will indicate the percentage of leaf destruction to which any chosen percentage of leaves showing markings corresponds. Thus, if 50 per cent. of the leaves show markings in one fumigation, approximately 29 per cent. of the leaf area of the plot will have been destroyed, thereby lowering the yield by approximately 9 per cent. Similarly, if 75 per cent. of the leaves show markings, nearly 55 per cent. of the leaf area will have been destroyed, with a corresponding reduction in yield of about 17 per cent. It is interesting to note also that a reduction in yield of 30 per cent. is indicated with a single 100-per cent. leaf destruction, or a double 52-per cent. leaf destruction, or a triple 35-per cent. leaf destruction.

These yield data are based on the average leaf destruction and average yield for plots of about twenty plants. The individual plants in any plot, however, vary greatly at different times, and also vary greatly from one

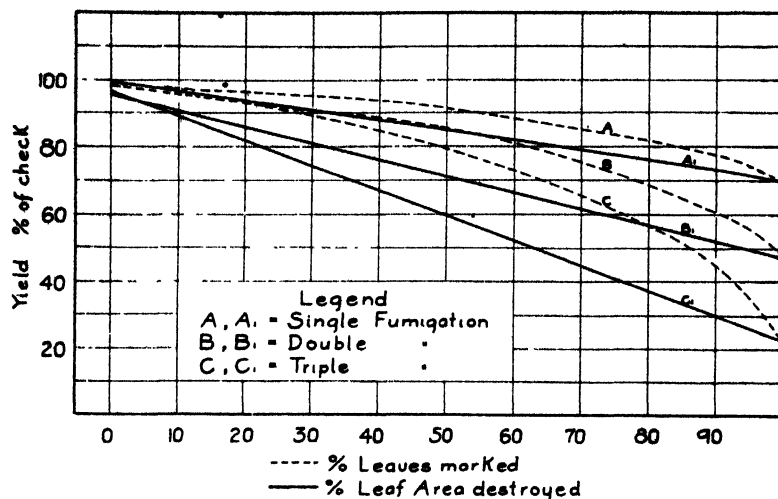


FIG. 4. Summary and comparison of the leaf-yield-destruction curves from figs. 2 and 3. Each curve represents one, two, and three fumigations on a single crop, and the leaf destruction is expressed as percentage of the total leaf area destroyed and as the percentage of the total number of leaves marked to any degree.

another in their ability to absorb sulphur dioxide. While this subject will be treated at length in a subsequent paper, it is desirable to call attention here to the fact of considerable differences between the percentages of markings on the different plants in a plot following a given fumigation. Table II

TABLE II

PERCENTAGE OF LEAF REDUCTION ON NINETEEN DIFFERENT PLANTS IN PLOT 51-2, FUMIGATED AUGUST 2, WITH AN AVERAGE LEAF DESTRUCTION OF 16.2%, AND AGAIN ON AUGUST 11, 1930, WITH AN ADDITIONAL AVERAGE LEAF DESTRUCTION OF 38%

PLOT 51-2, AUGUST 2				PLOT 51-2, AUGUST 11			
Row 1	Row 2	Row 3	Row 4	Row 1	Row 2	Row 3	Row 4
15	30	12	10	30	75	18	60
20		5	8	40		20	25
15	30	25	4	30	55	35	25
50	1	15	12	65	35	15	15
25	2	20	8	65	35	70	10

has been compiled to illustrate these differences. As will be noted in this table, the plants in plot 51-2 were fumigated twice during the same crop, on August 2 when the stems were 16.5 inches tall, and on August 11 when

the same stems averaged 19.8 inches in height. Comparison of the percentages of leaf destruction of different plants in the two fumigations will illustrate the variability in sensitiveness of the individual plants, as well as of the plants in the plot.

It seemed desirable to recalculate some of the yield data with this variability in mind. A number of plants were therefore selected at random from all parts of the field. These plants had all had a single fumigation on one crop in 1930. They were placed in seven groups, each group representing a different percentage of leaf destruction. The yield of each plant was then calculated, using the method already described for the plot yields. Table III presents the average leaf destruction and the average yield of each of these groups of plants, together with the standard deviations of the means of the yield. A comparison of the last two columns of the

TABLE III

RELATIVE YIELDS OF GROUPS OF ONCE-FUMIGATED INDIVIDUAL ALFALFA PLANTS SELECTED AT RANDOM FROM ALL PARTS OF THE FIELD, BASED ON PERCENTAGE OF LEAF DESTRUCTION, COMPARED WITH THOSE CALCULATED FROM EQUATION (1b); 1930 DATA

NO. OF PLANTS	LEAF DESTRUCTION		YIELDS IN PERCENTAGE OF CHECK	YIELDS CALCULATED FROM EQUATION (1b)
	RANGE	AVERAGE		
71	% Less than 1%	% 0.5	% $104 \pm 2.3^*$	99
68	5-10	8	$94 \pm 1.6$	97
52	20-25	23	$94 \pm 2.0$	93
50	40-45	42	$89 \pm 2.0$	87
29	55-60	57	$83 \pm 2.6$	83
62	70-75	73	$81 \pm 1.2$	78
31	85-95	92	$72 \pm 2.1$	72

\* Standard error of the mean.

table indicates the agreement between these yield values and those calculated from equation (1b). Both show very definitely that the decrease in yield of alfalfa is directly proportional to the percentage of leaf destruction.

C. YIELD OF ALFALFA AS INFLUENCED BY CHLOROTIC SULPHUR DIOXIDE MARKINGS.—Thus far in this study, the effects of only the acute type of markings have been considered. The conditions producing the chlorotic type are not very well understood, but they have been observed four or more days subsequent to short fumigations when the light intensity was low and

the concentration of sulphur dioxide was sufficiently high so that under conditions of high light intensity, severe acute markings would have resulted. Chlorotic markings also appear not infrequently after long-continued exposures to low concentrations of sulphur dioxide which are insufficient to produce markings of the acute type. Since chlorosis from a number of widely different causes is common on plants that have not been fumigated, it is frequently uncertain whether, in certain cases, the markings on plants which have been fumigated are due to sulphur dioxide or not.

**TABLE IV**  
**RELATIVE YIELD OF ALFALFA AS AFFECTED BY SULPHUR DIOXIDE MARKINGS OF THE CHLOROTIC TYPE**

No. OF PLOTS	TREATMENT	LEAF AREA MARKED		YIELD	STANDARD DEVIATION	STANDARD DEVIATION OF MEAN	YIELD CALCU- LATED FROM EQUATION (1b)
		ACUTE TYPE	CHLOROTIC TYPE				
		%	%	%	%	%	%
13	Fumigated	0.5	9.2	96.0	5.35	1.5	96.7

Table IV gives the yield of thirteen plots of alfalfa, upon the leaves of which numerous chlorotic markings were produced. While these data are insufficient to characterize the effect quantitatively, they indicate that the chlorotic lesions are *not more* effective in reducing yield than the same percentage of leaf area destroyed by the acute type of injury.

**D. YIELD OF ALFALFA AS AFFECTED BY CLIPPING TREATMENTS.**—In 1931, instead of destroying portions of alfalfa leaves with sulphur dioxide, the same range of percentages of leaf reduction as in the 1930 experiments was approximated by clipping off the leaves. These two treatments differed in the fact that, for a given amount of leaf destruction, whole leaves were removed by clipping and normal leaves left, whereas with fumigation, all the leaves were subjected to sulphur dioxide and the leaf destruction on individual leaves varied from zero to as much as 100 per cent. in some cases. Also the clipping treatment was applied uniformly to all of the plants, whereas with the sulphur dioxide treatments the individual plants of the plot showed widely varying percentages of leaf destruction, as indicated by table II. It is interesting to note, therefore, the close agreement in the yields between corresponding percentages destroyed by fumigation and by clipping.

The clipping treatment was applied to the second crop only. The other crops throughout the season were harvested as checks. Nine treatments

were carried out as follows: single, double, and triple clippings, in each of which about 20, 50, and 90 per cent. of the leaves were removed from each stem. In the case of double and triple clippings, the 20, 50, and 90 per cent. respectively of the leaves on each stem at the time of each clipping were removed. About forty-five plants from five different plots were subjected to each treatment. These treatments were carried out in the following order:

1. First of the triple clippings
2. First of the double clippings
3. Single clippings
4. Second of the triple clippings
5. Second of the double clippings
6. Third of the triple clippings

The triple clipping was begun about ten days after the harvest of the first crop, and was finished about seven days before the harvest of the second crop. The leaves from each plant were dried and weighed separately. The results are shown in table V, which gives the yield of the second crop with and without the leaves that were removed, and also the yield of the other three crops.

TABLE V

EFFECT ON YIELD OF IMMEDIATE AND OF TWO SUBSEQUENT CROPS OF ALFALFA OF CLIPPING OFF DIFFERENT PERCENTAGES OF LEAVES; CLIPPINGS MADE FROM SECOND CROP

NO. OF PLANTS	SECOND CROP TREATMENT		YIELD IN PERCENTAGE OF CHECK (FIRST CROP)				
			FIRST CROP	SECOND CROP		THIRD CROP	FOURTH CROP
	NO. OF CLIPPINGS	PERCENTAGE OF LEAVES REMOVED		WEIGHT	WEIGHT + WEIGHT OF LEAVES REMOVED		
45	1	20	100	94.2 $\pm$ 2.26*	98.7 $\pm$ 2.40*	104.2 $\pm$ 3.17*	96.0 $\pm$ 2.80*
42	1	50	"	80.3 $\pm$ 2.11	89.8 $\pm$ 2.34	97.5 $\pm$ 2.22	94.0 $\pm$ 2.87
45	1	90	"	68.0 $\pm$ 1.74	79.0 $\pm$ 1.89	93.5 $\pm$ 2.76	96.0 $\pm$ 2.68
48	2	20	"	85.5 $\pm$ 2.38	94.8 $\pm$ 2.47	100.6 $\pm$ 3.04	96.9 $\pm$ 2.54
45	2	50	"	70.9 $\pm$ 1.68	88.2 $\pm$ 1.74	91.9 $\pm$ 1.94	99.1 $\pm$ 2.46
46	2	90	"	57.8 $\pm$ 1.70	80.3 $\pm$ 2.50	81.6 $\pm$ 2.15	93.7 $\pm$ 2.97
48	3	20	"	70.4 $\pm$ 1.85	81.0 $\pm$ 2.12	99.6 $\pm$ 3.15	100.3 $\pm$ 2.73
47	3	50	"	61.3 $\pm$ 1.60	78.3 $\pm$ 1.94	93.5 $\pm$ 1.62	97.0 $\pm$ 2.25
46	3	90	"	41.5 $\pm$ 1.24	62.0 $\pm$ 1.77	73.8 $\pm$ 2.38	93.5 $\pm$ 2.15

\* Standard error of the mean.

In figure 5 the yield data for the clipping treatments have been plotted and the curves superimposed upon the fumigation yield curves from figure 2. The close agreement between these curves indicates that correspond-

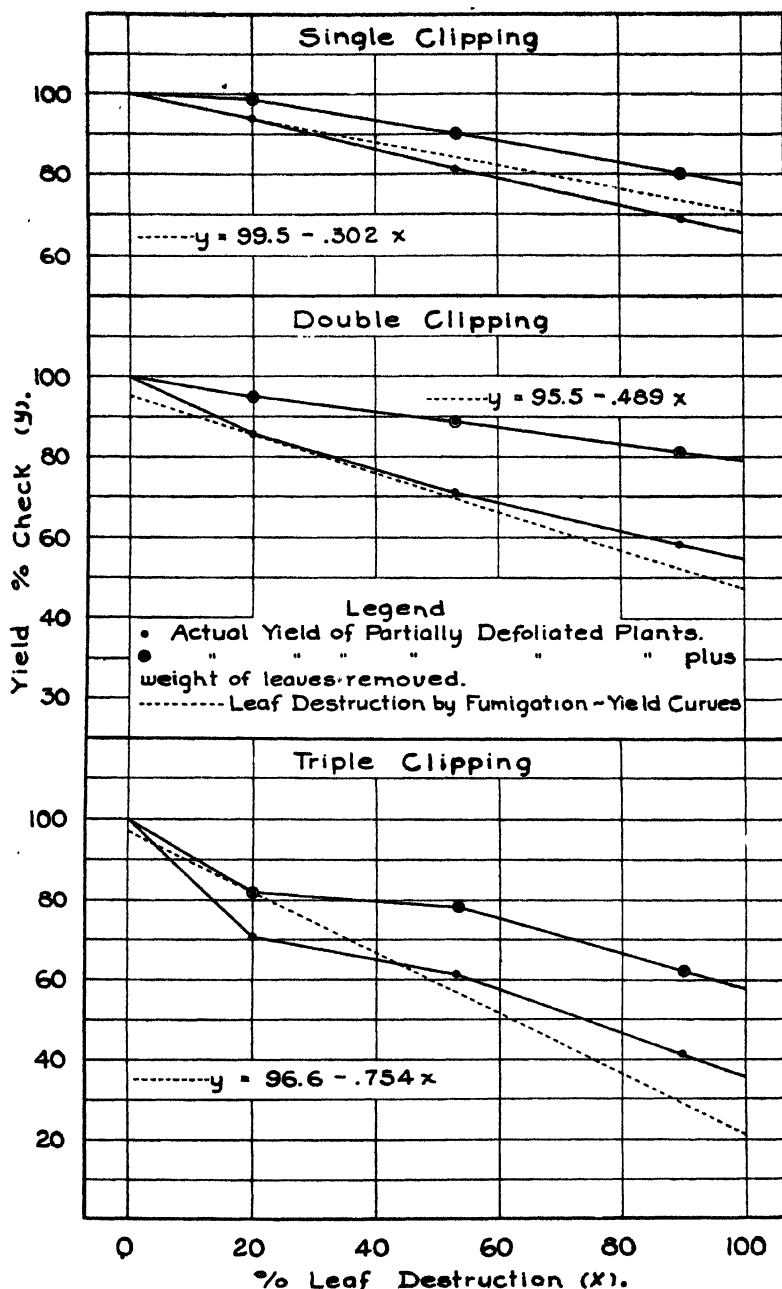


FIG. 5. Effect of clipping off various percentages of leaves one, two, and three times during the growth of a single crop on the yield of alfalfa. Treatments were applied on the second crop. The yield of each partially-defoliated crop is compared with the yield of the untreated crops from the same plots. Dotted lines are fumigation yield curves from fig. 2; 1931 data.

ing partial defoliations by clipping and by fumigation with  $\text{SO}_2$  give approximately the same reduction of yield. It should be noted that in the case of light fumigations there is little tendency to shed the injured leaves, and consequently the abscission yield data plus the weight of the leaves removed should agree more closely with the fumigation yield data at small percentages of leaf destruction. On the other hand, the abscission yield data without the weight of the removed leaves should agree more closely with the fumigation data showing large percentages of leaf destruction, on account of the tendency to shed the badly marked leaves. This agreement is shown in figure 5. The most extreme treatments appear to reduce the yield somewhat more in the case of fumigation than in the case of corresponding defoliation by clipping. The difference, although small, is possibly due to an added percentage of green tissue which is lost to the plant when the badly burned leaves are shed. The remarkable concordance between the fumigation and abscission treatments indicates that sulphur dioxide affects the alfalfa plant essentially and proportionately by removing leaf tissue. If any other effect is produced, it is of a definitely minor character.

E. EFFECTS OF PARTIAL DEFOLIATION ON SUBSEQUENT CROPS.—Table V also indicates that the yield on the third and fourth crops, subsequent to clipping the leaves on the second crop, is nearly normal in the case of the less severe treatments. The yield of the third crop, however, following the triple 90 per cent. clipping on the second crop, is only about 74 per cent. of normal, and the yield of the fourth crop is only about 94 per cent. of normal. In the case of the double 90 per cent. clipping on the second crop, these values are 82 per cent. normal for the third crop and 94 per cent. normal for the fourth. This effect, which possibly is to be explained by root depletion during second-crop recovery, has also been similarly noted in the case of heavily fumigated treatments (table VI). The fumigation

TABLE VI

INFLUENCE OF SEVERITY OF SULPHUR DIOXIDE FUMIGATIONS UPON RELATIVE YIELDS OF THE SUBSEQUENT CROP OF ALFALFA

NO. OF PLOTS	AVERAGE YIELD OF FUMIGATED CROP		AVERAGE YIELD OF SUBSEQUENT UNTREATED CROP
	RANGE	AVERAGE	
	%	%	%
28	80-90	85.7	99.2 $\pm$ 3.7*
10	70-80	76.6	92.2 $\pm$ 6.7
8	60-70	65.8	81.3 $\pm$ 4.5
5	40-60	52.2	72.0 $\pm$ 7.5

\* Standard error of the mean.

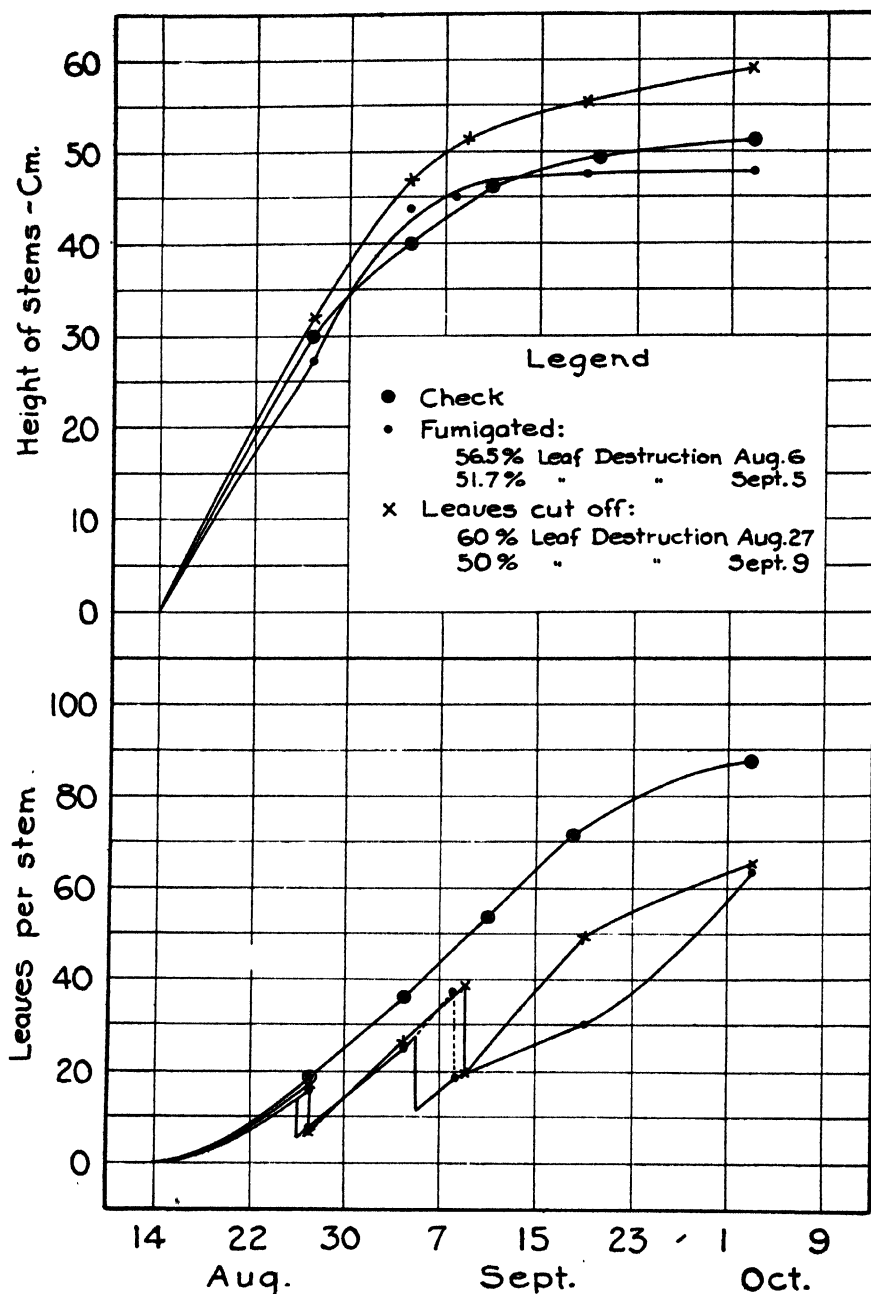


FIG. 6. Effect of leaf destruction by sulphur dioxide fumigation and by corresponding leaf clipping on the growth and formation of new leaves on individual stems of alfalfa.

treatments, however, were not especially planned to reveal this effect, as were the abscission treatments, and data are available on only one crop subsequent to the fumigation.

In table VI the 1930 yield data of the fumigated plots have been arranged in groups of 10 per cent. range, except for the fourth entry, in which it is 20 per cent. Tables V and VI indicate that the rates of recovery from abscission and fumigation treatments are nearly identical, and that the subsequent crop is practically normal if the treatment of the crop which precedes it does not reduce the yield more than about 15 per cent.

F. GROWTH AND LEAF PRODUCTION OF INDIVIDUAL ALFALFA STEMS.—To determine the behavior of alfalfa plants following fumigation and clipping treatments, twenty comparable vigorous stems were twice subjected to each treatment and their subsequent growth was compared with that from forty corresponding untreated stems. In this experiment the numbers of leaves were counted and the heights of the stems were measured at intervals throughout the growth period. These data are presented in figure 6. At harvest time the average dry weight of the fumigated stems was 9.8 grams, of the clipped stems, 10.1 grams, and of the untreated stems, 16.2 grams. While these observations have not been sufficiently extensive in character to have quantitative significance, they indicate that recovery of the plants from fumigation treatments is similar to that from abscission treatments, and that in both cases the subsequent production of leaves and growth of stems is comparable with that of untreated stems.

### Summary

1. Sulphur dioxide lesions on alfalfa are of two types, acute and chlorotic. The former shows characteristic bleached interveinal and marginal areas in otherwise normally appearing leaves; the latter exhibits a more or less yellowed and mottled appearance and is similar to chlorosis as produced by a number of other causes.

2. The reduction in yield of alfalfa subjected to a single sulphur dioxide fumigation of one crop is in direct proportion to the percentage of leaf area destroyed. The stage of growth at which the fumigation occurs does not appear to influence the result, at least within the range of 25–75 per cent. of the total growth period of the plant.

3. If a crop is fumigated more than once, assuming at least a week to elapse between fumigations each of which produces the same percentage of leaf destruction, the reduction of the yield is also in proportion to the number of fumigations.

4. The reduction in yield is not a linear function of the number of leaves marked, indicating that the uninjured portions of the leaves continue to function in spite of bleached areas.

5. The yield-leaf-destruction curves all closely approach 100 per cent. yield at 0-per cent. leaf destruction, indicating that the gas does not reduce the yield unless it produces visible effects.

6. The reductions of yield caused by sulphur dioxide fumigation can be closely duplicated by clipping off from normal plants an amount of leaf tissue equivalent in area to that destroyed by fumigation.

7. Like acute markings, chlorotic sulphur dioxide markings appear to lower the yield in direct proportion to the percentage of leaf tissue visibly affected.

8. A severe defoliation treatment, either by fumigation or by clipping, which lowers the yield of the treated crop extensively, also reduces the yield of the subsequent untreated crop appreciably, and may even be felt slightly in the second subsequent untreated crop.

9. The subsequent growth of new leaves, and the elongation of the stems following a partial defoliation, proceed at the same rate, regardless of whether the leaf destruction was accomplished by sulphur dioxide fumigation or by clipping.

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# PHOTOSYNTHESIS IN VARIOUS PORTIONS OF THE SPECTRUM<sup>1</sup>

G. RICHARD BURNS

(WITH THREE FIGURES)

## Introduction

The work on photosynthesis in various parts of the spectrum has been recently reviewed (5, 1, 9), and need not be repeated here except to call attention to the experimental methods and the results of the work of WARBURG and NEGELEIN (6), WURMSER (7, 8), and BRIGGS (1). Using a unicellular alga, *Chlorella vulgaris*, WARBURG and NEGELEIN found, under the conditions of their experiments, that for the reduction of one molecule of carbon dioxide, 4.4 quanta were necessary in the red, 4.3 in the green, and 5.1 in the blue. WURMSER found a higher value in the green, and concluded that photosynthesis does not follow the photochemical equivalence law. BRIGGS, working with land plants, agrees with WARBURG rather than with WURMSER. In the blue his results are similar to those of the writer in the blue-violet. WARBURG and WURMSER, using water suspensions of algae, were able to obtain a condition of complete absorption and very low reflection. BRIGGS, using cut leaves from land plants, had incomplete absorption. All corrected for respiration by adding the dark-rate respiration to their apparent rate of photosynthesis. This expedient is necessary if one wishes to determine the absolute quantum yields. In determining relative quantum yields, the writer's method makes it necessary to assume only that respiration is the same under two light intensities that are causing the same amount of photosynthesis.

The experiments reported in this paper were undertaken from a somewhat different point of view than the preceding. The chief object was to determine how efficiently certain trees could use various portions of the spectrum in photosynthesis. For this reason interest centered primarily on the incident radiation rather than on the absorbed radiation, and no attempts were made to measure absorption and reflection. To determine the photosynthetic efficiency on the basis of incident light, the plants could not be allowed to grow for a long time in colored light, as the effect of different wave lengths of light on the physical form of the plant entirely masks the photosynthetic effect (2, 4). Single leaves or groups of leaves could not be used, as the removal of any portion of a plant affects the rate of photosynthesis or of respiration. Narrow-leaved plants were used since

<sup>1</sup> Published with the consent of the Director of the Vermont Agricultural Experiment Station.

with broad-leaved plants a slight movement of the leaves may result in a considerable change in their effective area and reflection. The narrow-leaved plants used had the additional advantage that they were dark in color and thus absorbed a large portion of the incident radiation. The main features of the experimental method were: careful preliminary adjustment of the plant to the light intensity to be employed; use of light of constant and known intensity so adjusted that the amount of photosynthesis in each pair of determinations was the same; and, finally, measurement of the amount of photosynthesis from the decrease in carbon-dioxide concentration in the air surrounding the plant.

### Apparatus

PLANTS USED.—White pine (*Pinus strobus*), Norway spruce (*Picea excelsa*), and Engelmann spruce (*Picea engelmanni*) were used, four-year-old nursery stock of regular shape and similar size being selected. They were potted and placed in a greenhouse three months before the work was begun.

The experiments were carried out in a constant-temperature room cooled by cold air and kept at the necessary temperature by a thermostatically controlled, electrical "space heater." The temperature at the plant was  $28^{\circ} \pm 0.5^{\circ}$  C. unless otherwise stated. The temperature coefficient at this temperature, with the light intensities used, is one.

The light sources were four 1000-watt, 115-volt, projection type Mazda lamps operated at 110 volts. This type had the advantage of giving more light and having smaller bulbs than the ordinary lamp. The rate of decay in visible radiation was about 1 per cent. per hour at 110 volts and 2 per cent. at 115 volts. Line voltage fluctuations were virtually eliminated by the use of a General Electric miniature automatic induction voltage regulator. If the voltage fell to 109.5 or rose to 110.5, this instrument returned it to 110.0. This made the maximum deviation in light intensity about 1 per cent. and the average variation about 0.5 per cent. It was essential that some means of regulating the voltage be used, inasmuch as a 5.5 per cent. change in voltage resulted in a 16 per cent. change in the intensity of the radiation between 1150  $m\mu$  and 400  $m\mu$ , and a 28 per cent. change between 550  $m\mu$  and 400  $m\mu$ . To avoid reflected radiation, the bulb was surrounded with a blackened shield having a 10-cm. aperture toward the plant. A water filter was placed directly outside this aperture at a distance of 6 cm. from the filament. The water filter consisted of a 6-inch brass ring, 1 inch wide, with 3-mm. plate glass windows ground on and held in place by clamps. Tap water was circulated through these at 2.5 ft.<sup>3</sup> per hour. It removed infra-red radiation to 1150  $m\mu$ . Because of the large area to be illuminated and the high intensity required, it was not pos-

sible to use anything approaching monochromatic light. Glass color filters were used which isolated the red, red-yellow, and blue-violet portions of the visible spectrum (fig. 1). These were fastened to the water cells. The blue-violet filters sometimes broke in spite of protection afforded by the water cell.

The radiation intensity was determined by a linear thermopile fitted with a 10-cm. water cell with quartz windows. A balanced-current method



FIG. 1. Transmission spectra of filters with lamp at 110 volts. 1. Blue-violet + water filter. 2. Red-yellow + water filter. 3. Red + water filter. 4. Water filter. 5. No filter. 6. Sunlight (visible only, greatly reduced).

was employed, using a galvanometer as a zero instrument (3). The 10-cm. water cell insured more accurate readings by absorbing re-radiation from the hot glass filters; and, by absorbing an additional amount of the infrared, made possible a more accurate calculation of the amount of visible radiation. The conversion factors necessary to determine the total radiation and the light intensity were found both by direct and by spectrometric

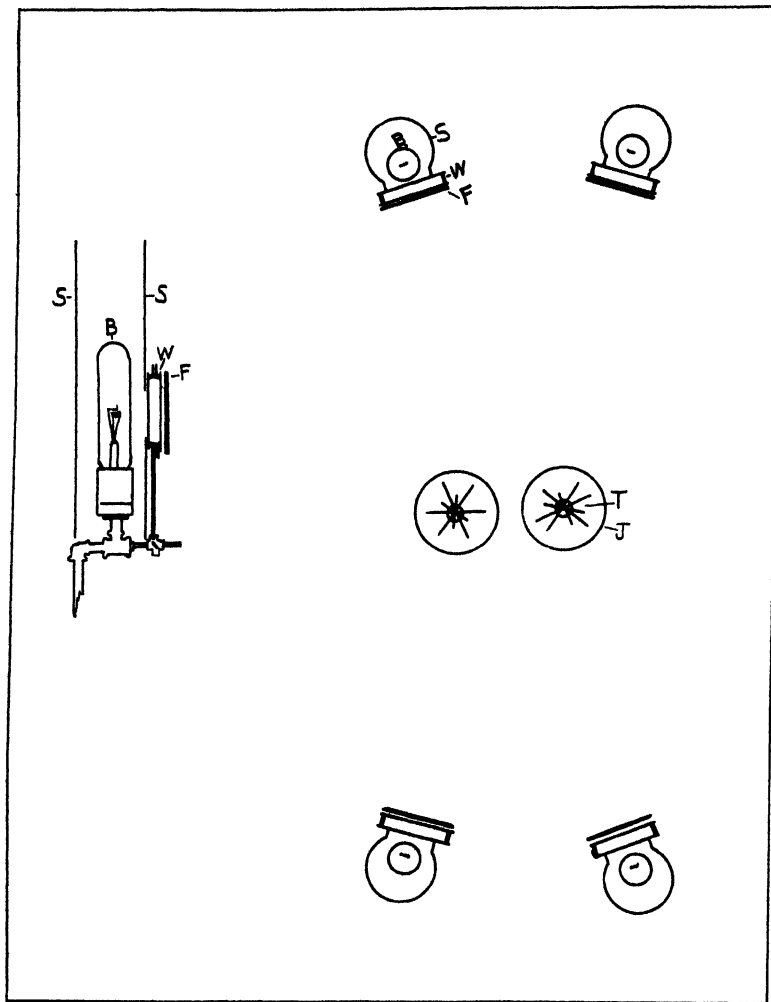


FIG. 2. Arrangement of lights, top and side view. B, bulb; S, shield; W, water filter; F, color filter; T, tree; J, bell-jar.

measurements. The factors by the two methods differed by not more than 0.4 per cent. The transmission spectra of the water cells and the color

filters were measured by means of a Hilger spectrometer (D 77) and thermopile.

The four lights were placed at the four corners of a rectangle, with one plant near the center and the second plant as close to it as possible (fig. 2). The plants were always set in exactly the same position, and when the lights were moved they were moved directly towards or away from the plant near the center. The radiation intensity was measured at the point that had been occupied by the center of the crown of the plant. This gave a value that is about 1 per cent. low, owing to the fact that some of the leaves are in front of this point and some are behind it. The lights and the trees were on the same level.

Since the relative amount of photosynthesis was determined by carbon-dioxide assimilation, generally considered the most accurate criterion, it was necessary to have the crown of the tree in a gas-tight container. This was done by fitting a ground-glass plate around the lower part of the stem and sealing a bell-jar on the plate with stop-cock grease. The bell-jar was equipped with a fan, so arranged that it would not shade the tree yet would provide adequate mixing of the gas before analysis. The smallest possible bell-jar was used, as this made the percentage decrease in carbon dioxide greater and thus increased the accuracy of the results. The bell-jars were used with the inside surface wet with a dilute soap solution. This was the only method found which would prevent the formation of a fog by the transpired moisture from the plant. The soap film was found to have no measurable effect on the carbon-dioxide concentration. The light transmission of the wet bell-jars was measured with the jar whirling rapidly with an excentric motion. In table I are presented the volumes and transmission data for the bell-jars used.

The carbon-dioxide determinations were made with a modified Haldane

TABLE I

BELL-JAR	RUNS USED		VOLUME	TRANSMISSION
1	NS	1 1-68	8,850	89
1	P	1 All	..	..
2	NS	2 69-73	10,350	84
3	NS	2 1-68	10,750	88
4	ES	4 All	2,700	89.5
5	ES	3 All	5,150	90
5	P	4 All	.....	.....
5	P	3 41-43	.....	.....
6	P	3 1-41, 44-70	4,600	89
7	NS	1 69-73	5,260	88

gas analysis apparatus with which it was possible to determine the percentage of carbon dioxide to 0.005.

### Experimental procedure

The crown of the tree was carefully inserted through a hole in a ground-glass plate and the opening around the stem closed with a rubber stopper and grafting wax. The whole was then set on a base which supported the pot and the plate so that they could not move. The tree was then placed outdoors in light of approximately the same intensity as was to be used in the experiment and left for one week. This was a most important precaution, as in the case of the spruces a decrease as great as 15 per cent. resulted in the amount of carbon dioxide used when the tree was exposed to bright sunlight the day before the determination. In the case of white pine, which is more sensitive to such changes, the minimum light requirement, that is, the light intensity at which photosynthesis and respiration balance, was found to have decreased 50 per cent. after the tree had been in dull light for six days.

For a determination, the plant was brought into the constant temperature room, the bell-jar sealed on and tested for leaks, carbon dioxide introduced until its concentration was about 1 per cent., and the plant placed in a light intensity near its minimum light requirement. After half an hour the gas was well mixed, an accurate analysis made, and the plant placed in the desired light intensity for exactly two hours. At the expiration of this time it was withdrawn, the radiation intensity measured, and the final gas analysis made. As a rule two plants were used at the same time and two sets of determinations were made each day. After the last analysis of the day the plants were again placed outdoors in the dull light.

As would be expected, it was found to be important that the initial amount of carbon dioxide be kept uniform. Initial concentrations as high as 2 per cent. and as low as 0.1 per cent. were found to make detectable differences in the minimum light requirement. The decrease in concentration of carbon dioxide during a determination is the amount that has been used by photosynthesis minus the amount given off by respiration. Since it was not possible to determine the amount of respiration, the light intensities were so adjusted that the total change in carbon dioxide was about the same in each pair of determinations, in order that the efficiency of the two kinds of light could be judged from their relative intensities. Under these conditions it was safer to assume that respiration, and therefore photosynthesis, was the same. The method had the additional advantage that the ranges of available carbon-dioxide concentrations were the same. The small corrections necessary to find the light intensities that would give exactly equal decreases in carbon-dioxide concentration were determined by

making a graph of the light intensities against the decrease in carbon dioxide (fig. 3). To eliminate errors due to the plants being imperfectly adjusted to the low light intensity, the determinations with colored light were run first one day and second the next. If the photosynthetic efficiency were still changing, the two sets of determinations would not agree but their average would be close to the true value.

### Results

In the following tables the run number is the serial number of the day on which the determination was made, and the number of primes indicates whether it was the first, second, or third determination on the tree that day. The second column gives the number of the tree used: *P* for white pine, *NS* for Norway spruce, and *ES* for Engelmann spruce. The next column gives the filters used; the next, the decrease in carbon-dioxide concentration expressed as percentage of total gas; then the total radiation in calories per  $\text{cm}^2$  per run  $\times 10$ . The column headed "light" is the amount of radiation shorter than 720  $\text{m}\mu$  in calories per  $\text{cm}^2$  per run  $\times 10$  (70 in these readings is about equal to 1 per cent. full sunlight). The values are not corrected for transmission of the bell-jars. The column "efficiency based on radiation" gives the number of parts of total radiation (see fig. 1) through the 1-inch water filter necessary to produce the same decrease of carbon dioxide as 100 parts of the radiation being studied. The next column is the same but based on the light intensity and not the total radiation. For example, in table II, white pine 3 on an afternoon run in the 43rd experiment with the radiation from the bulbs filtered through 1 inch of water and the blue-violet filter reduced the percentage of carbon dioxide in the bell-jar by 0.065 and 0.060, as shown by duplicate readings. During this time the total radiation received by the plant was  $58.8 \text{ cal. per cm}^2 \times 10$ , and the light, that is, the amount of radiation shorter than 720  $\text{m}\mu$ , was  $40.0 \text{ cal. per cm}^2 \times 10$ . When the blue-violet filter was removed and the lights moved farther away, however, the same tree in the noon run (43") reduced the percentage of carbon dioxide in the bell-jar by 0.085, 0.080. The total radiation in this determination was 57.2 and the light 19.2. The amounts of radiation and light necessary to reduce the percentage of carbon dioxide by 0.0625, determined by means of a graph (fig. 3), were 48.0 and 16.2 respectively. The calculated value for the radiation through the 1-inch water filter multiplied by 100, divided by the amount of the blue-violet radiation, gives 81.7, the efficiency of the blue-violet radiation. That is, 81.7 parts of radiation through the 1-inch water filter were equal to 100 parts of radiation through the 1-inch water filter plus the blue-violet filter. Taking as values the light intensities rather than the total radiation intensities, the efficiency of the blue-violet is 40.4. These figures are based

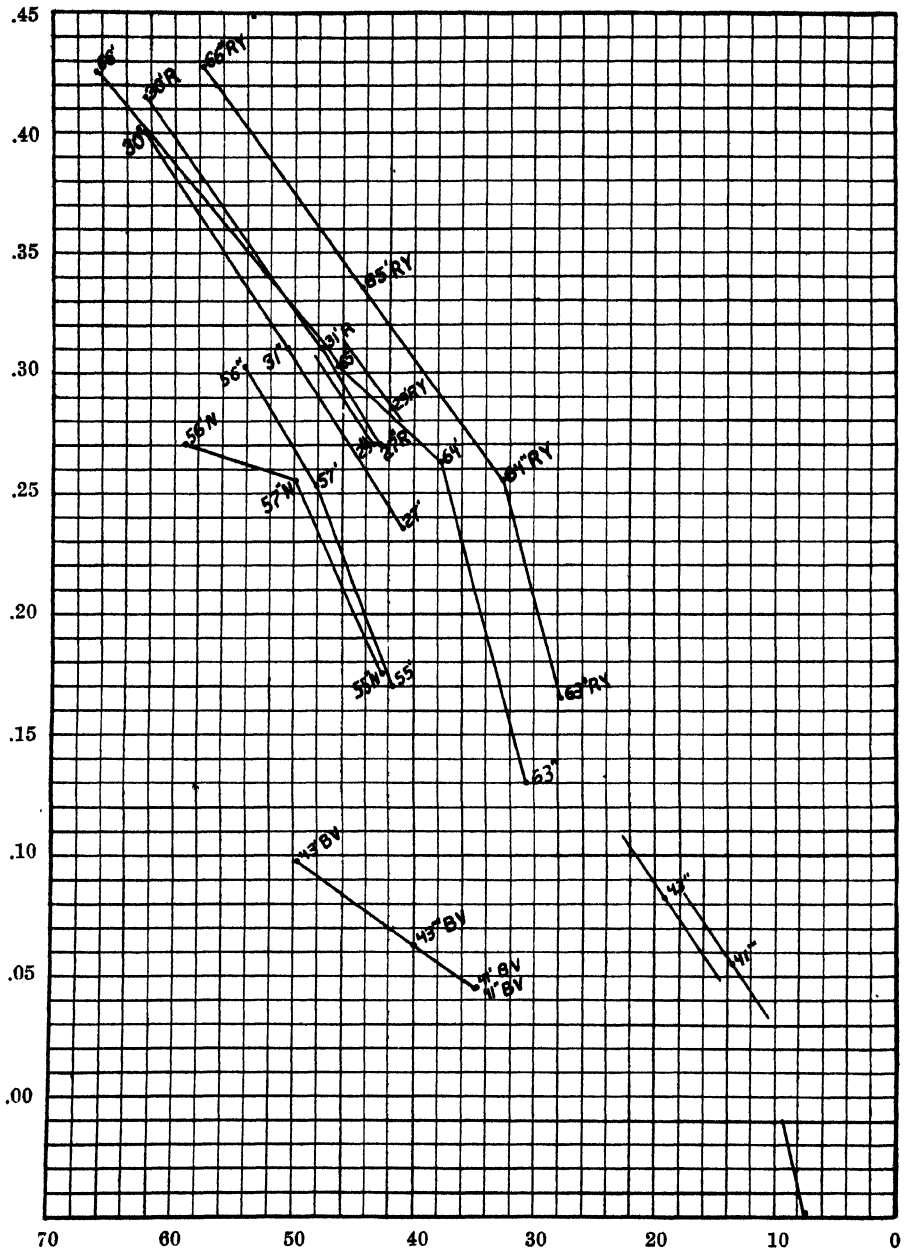


FIG. 3. Curves of decreased percentage of carbon dioxide plotted against light intensity. White pine 3. N = no filters. Run numbers alone indicate 1-in. water filters. R = 1-in. water filter + red filter. RY = 1-in. water filter + red-yellow filter. BV = 1-in. water filter + blue-violet filter.

on the assumption that radiation of wave length longer than 720  $m\mu$  is useless in photosynthesis. This is a major assumption, of course, and practically without direct experimental proof. There are, however, many things indicating that this is true. An investigation of the exact long wave-length limit of photosynthesis will be started in the near future. The final column gives the sum of the errors that would result from an error of 0.01 per cent. (twice the actual error) in the gas analysis, and the probable error in correcting the light intensity from the graph. In addition to this there are errors of 1 per cent. in measuring the radiation intensity, 0.5 per cent. from variation of the radiation intensity, and a constant error in calculating the radiation and light intensity.

In the runs in which the blue-violet filter was used, the voltage was raised to 115 in order to increase the amount of energy in this portion of the spectrum. The runs with the water filter alone were at 110 volts. Owing to the low intensity of the blue-violet, the lights were much closer to the plant, and this slightly increased the error in measuring the light intensity. To reduce the white light to a comparable intensity, it was necessary to use smoked screening in front of the water cells in addition to moving the lights farther away. It should be noticed that the change in efficiency in white pine 4 is due to a loss of efficiency in white light, rather than to any change in the blue-violet. For this reason white pine 3 was given a 7-hour exposure to the blue light in run no. 42. In this case no such marked change was observed.

The efficiency of the red-yellow would be the same regardless of the long wave-length limit of photosynthesis, since these filters transmit the same proportions of infra-red as do the 1-inch water cells. White pine 4 gives the same results here that it did with the blue-violet. With white pine 3 a slight fog was formed as noted. This throws the values off by as much as 10 per cent. Determinations 65 and 66 are probably the most accurate, as the 64' falls out of place on the graph.

The determinations 58 to 60 inclusive, on white pine and Engelmann spruce, were made after the trees had been placed in a small cubical greenhouse glazed with 80 panes of the red filter glass and left for three weeks in full sunlight. The last ten days of this time were almost cloudless. The customary acclimation period was omitted. It will be noticed that this treatment resulted in a considerable change in the relative efficiencies.

These determinations show that infra-red radiation longer than 1100  $m\mu$  is slightly detrimental to photosynthesis when the plant is at a sufficiently high temperature. It should be noted that the temperature coefficient of photosynthesis at 28° C. is so near 1 that no effect of temperature can be detected in these measurements. It is also interesting to note

TABLE II  
BLUE-VIOLET

RUN NO.	TREE NO.	FILTERS	DECREASE IN CO <sub>2</sub>		TOTAL RADIATION	LIGHT	EFFICIENCY OF BLUE-VIOLET BASED ON		EXPERIMENTAL ERROR
			I	II			RADIATION	LIGHT	
33'	ES 4	1"	% 0.110	% 0.110	cal. 66.6	cal. 22.4	%	%	% 3.0
36'	ES 4	1"-BV	0.050	0.050	69.2	47.1			
36"	ES 4	1"	0.060	0.060	57.4	19.3*	71.0	35.2	3.0
37'	ES 4	1"-BV	0.070	0.065	80.0	54.4			
37"	ES 4	1"	0.055	0.060	52.3	17.6	67.9	33.6	3.0
37'''	ES 4	1"-BV	0.045	0.045	66.2	45.0			
41'	P 3	1"-BV	0.045	0.045	51.2	34.8	71.0	35.2	3.0
41"	P 3	1"-BV	0.050	0.040	51.3	34.9	70.5	34.8	4.5
41'''	P 3	1"	0.055	0.055	40.3	13.6	73.0	36.2	6.0
42	P 3	1"-BV			44.1	30.0			
43'	P 3	1"-BV	0.095	0.100	73.0	49.7			
43"	P 3	1"	0.085	0.080	57.2	19.2	86.7	42.7	3.0
43'''	P 3	1"-BV	0.065	0.060	58.8	40.0			
38'	P 4	1"-BV	0.100	0.100	64.0	43.5	81.7	40.4	3.0
38"	P 4	1"	0.065	0.065	42.7	14.4	84.0	41.5	5.0
38'''	P 4	1"-BV	0.115	0.115	64.8	44.1	89.0	44.0	5.0
38'''	P 4	1"	0.080	0.075	55.9	18.8	100.0	49.5	4.0
39"	P 4	1"	0.100	0.095	68.3	22.9	112.0	55.5	4.0

\* Fog 37" value used.

TABLE III  
RED-YELLOW

RUN NO.	TREE NO.	FILTERS	DECREASE IN CO <sub>2</sub>		TOTAL RADIATION	LIGHT	EFFICIENCY OF RED-YELLOW BASED ON		EXPERIMENTAL ERROR
			I	II			RADIATION	LIGHT	
			%	%	cal.	cal.			%
29'	P 4	1"-RY	0.355	0.340	134.0	45.4			
29''	P 4	1"	0.335	0.335	128.0	43.4	99.3	98.7	2.0
63'	P 4	1"-RY	0.135	0.130	78.7	26.7			
63''	P 4	1"	0.140	0.140	75.5	25.4	92.2	91.6	3.0
64'	P 4	1"	0.240	0.240	111.0	37.4			
64''	P 4	1"-RY	0.235	0.235	96.3	32.7	112.3	111.7	2.0
65'	P 4	1"-RY	0.300	0.305	123.0	41.9			
65''	P 4	1"	0.265	0.270	135.0	45.6	123.6	122.9	2.0
66'	P 4	1"	0.305	0.305	187.0	62.9			
66''	P 4	1"-RY	0.400	0.395	164.0	55.6	151.3	150.5	3.0
29'	P 3	1"-RY	0.290	0.280	123.0	41.9	110.1	109.5	2.0
29''	P 3	1"	0.270	0.270	129.0	43.4*			
63'	P 3	1"-RY	0.165	0.165	82.2	27.8*			
63''	P 3	1"	0.130	0.130	91.3	30.7*	111.2	110.2	5.0
64'	P 3	1"	0.265	0.260	111.0	37.3			
64''	P 3	1"-RY	0.255	0.255	96.2	32.6	112.0	111.0	2.0
65'	P 3	1"-RY	0.335	0.335	131.0	44.4			
65''	P 3	1"	0.305	0.300	138.0	46.4	117.5	116.8	1.5
66'	P 3	1"	0.430	0.420	197.0	66.5			
66''	P 3	1"-RY	0.425	0.430	170.0	57.7	117.0	116.3	1.0
69'	NS 1	1"-RY	0.400	0.400	193.0	65.4			
69''	NS 1	1"	0.440	0.440	232.0	78.2	106.0	105.3	3.0
72'	NS 1	1"							
72''	NS 1	1"-RY	0.375	0.370	159.0	53.7			
73'	NS 1	1"-RY	0.435	0.435	202.0	68.6			
73''	NS 1	1"	0.420	0.420	201.0	67.8	104.7	104.0	3.0
69'	NS 2	1"-RY	0.210	0.205	191.0	64.7			
69''	NS 2	1"	0.245	0.245	226.0	76.2	102.6	102.0	4.0
72'	NS 2	1"	0.225	0.225	180.0	60.6			
72''	NS 2	1"-RY	0.200	0.200	155.0	52.4	106.3	106.0	4.0
73'	NS 2	1"-RY	0.220	0.215	196.0	66.5			
73''	NS 2	1"	0.220	0.215	201.0	67.7	102.5	102.0	4.0

\* Slight fog.

TABLE IV  
Red

RUN NO.	TREE NO.	FILTERS	DECREASE IN CO <sub>2</sub>		TOTAL RADIATION	LIGHT CAL. PER CM. <sup>2</sup> × 10	EFFICIENCY OF RED-YELLOW BASED ON		EXPERIMENTAL ERROR
			I	II			RADIATION	LIGHT	
			%	%	cal.	cal.			%
27'	P 3	1"	0.235	0.235	122.0	40.9			
27''	P 3	1"-Red	0.270	0.270	294.0	42.9	67.5	107.0	2.0
30'	P 3	1"-Red	0.415	0.415	298.0	62.5			
30''	P 3	1"	0.800	0.795	373.0	125.2	65.0	104.5	6.0
31'	P 3	1"-Red	0.310	0.310	227.0	47.8			
31''	P 3	1"	0.310	0.310	150.0	50.6	65.9	106.0	2.0
27'	P 4	1"	0.300	0.300	132.0	44.2			
27''	P 4	1"-Red	0.295	0.295	197.0	41.4	65.5	105.4	2.5
30'	P 4	1"-Red	0.450	0.445	285.0	59.8			
30''	P 4	1"	0.765	0.750	346.0	116.0	65.5	105.4	6.0
31'	P 4	1"-Red	0.380	0.390	226.0	47.6			
31''	P 4	1"	0.385	0.380	150.0	50.6	67.0	107.0	2.0
58'	P 1	1"-Red	0.400	0.400	242.0	50.7			
58''	P 1	1"							
59'	P 1	1"-Red	0.300	0.305	180.0	37.9			
59''	P 1	1"	0.290	0.280	132.0	44.4	77.0	123.5	1.5
60'	P 1	1"	0.350	0.350	160.0	53.8			
60''	P 1	1"-Red	0.305	0.305	196.0	41.2*	71.5*	114.8*	
23'''	ES 4	1"-Red	-0.005	-0.005	42.2	14.2			
23'''	ES 4	1"-Red	0.000	0.000	66.3	13.9	65.7	106.0	5.0
24'	ES 4	1"	0.150	0.155	95.5	32.1			
24''	ES 4	1"-Red	0.145	0.145	146.0	30.7	64.0	102.0	1.5
23'''	ES 3	1"	0.005	0.000	42.8	14.4			
23'''	ES 3	1"-Red	0.000	0.000	64.8	13.6	64.2	103.0	7.0
24'	ES 3	1"	0.155	0.150	100.0	33.7			
24''	ES 3	1"-Red	0.155	0.160	155.0	32.2	65.9	106.0	2.0
58'	ES 3	1"-Red	0.390	0.390	247.0	52.4			
58''	ES 3	1"							
59'	ES 3	1"-Red	0.305	0.305	191.0	40.3			
59''	ES 3	1"	0.290	0.290	138.0	46.4	75.4	120.0	1.0
60'	ES 3	1"	0.395	0.395	172.0	57.7			
60''	ES 3	1"-Red	0.365	0.365	211.0	44.7	76.5	123.0	1.5

\* Fog value low.

TABLE V  
INFRA-RED

RUN NO.	TREE NO.	FILTERS	DECREASE IN CO <sub>2</sub>		TEMPERATURE	TOTAL RADIATION CAL. PER CM. <sup>2</sup> X 10	LIGHT CAL.	EFFICIENCY OF INFRA-RED + LIGHT BASED ON LIGHT		EXPERIMENTAL ERROR
			I	II						
55'	P 3	1"	% 0.175	% 0.165	29.5	cal. 124.0	cal. 41.8	{	92.3	1.5
55''	P 3	None	0.170	0.180	29.5	372.0	42.6			
56'	P 3	None	0.270	0.270	30.5	514.0	59.1	{	91.7	1.0
56''	P 3	1"	0.305	0.300	30.0	161.0	54.1			
57'	P 3	1"	0.250	0.255	29.5	143.0	48.1	{	97.6	1.0
57''	P 3	None	0.255	0.255	29.5	434.0	49.9			
55'	P 4	1"	0.205	0.205	29.5	127.0	42.6	{	91.7	1.5
55''	P 4	None	0.220	0.215	29.5	397.0	45.7			
56'	P 4	None	0.315	0.315	30.5	531.0	61.0	{	87.3	1.0
56''	P 4	1"	0.345	0.345	30.0	169.0	57.0			
57'	P 4	1"	0.275	0.270	29.5	144.0	48.7	{	90.1	1.0
57''	P 4	None	0.270	0.260	29.5	450.0	51.7			
13''	NS 1	1"	0.000	0.000	27.0	29.4	9.9	{	92.8	10.0
44'	NS 1	1"	0.150	0.160	28.0	158.0	53.3			
44''	NS 1	None	0.125	0.135	31.0	453.0	52.1	{	92.5	2.5
45'	NS 1	None	0.340	0.340	33.5	974.0	112.0			
45''	NS 1	1"	0.395	0.395	33.5	332.0	112.0	{	87.5	1.0
53'	NS 1	None	0.210	0.210	29.5	561.0	64.5			
53''	NS 1	1"	0.235	0.225	28.5	189.0	63.5	{	92.2	2.0
54'	NS 1	1"	0.220	0.225	29.5	178.0	60.0			
54''	NS 1	None	0.220	0.230	29.5	551.0	63.3	{	95.4	2.0
14'	NS 2	1"	0.000	0.000	27.0	29.8	10.0			
44'	NS 2	1"	0.125	0.120	28.0	155.0	52.1	{	93.0	10.0
44''	NS 2	None	0.110	0.110	31.0	448.0	51.5			
45'	NS 2	None	0.285	0.300	33.5	914.0	105.0	{	96.6	3.0
45''	NS 2	1"	0.320	0.315	33.5	324.0	109.0			
53'	NS 2	None	0.165	0.170	29.5	503.0	58.0	{	86.8	1.5
53''	NS 2	1"								
54'	NS 2	1"	0.210	0.210	29.5	171.0	57.7	{	86.8	2.5
54''	NS 2	None	0.190	0.195	29.5	537.0	61.7			

that with pine the adverse effect of the infra-red is more noticeable in the early morning run than in the later one.

The following summary gives the amount, in calories, of white light (720–390 mμ) necessary to give the same percentage decrease in carbon-dioxide concentration as 100 calories of the light in question:

	NS 1	NS 2	ES 3	ES 4	P 3	P 4	ES 3	P 1
							ACCLIMATED	
Blue-violet .....				34.4	38.8	39.5	..	..
Red-yellow . . . . .	106	103			116.0	....	..	.....
Red .....			106	102.0	106.0	...	121	123
Infra-red and white	91	92			92.0	91.0	..	

### Relative quantum yields

The effective area of white pine 3 was calculated by photographing the tree from the positions of the four lights, enlarging these, and cutting out the unexposed portions. The weight of these was then compared with the weight of a known area photographed and enlarged in the same manner. Suitable precautions were taken to avoid the creeping of the background over the image. The average of the four areas was  $105 \pm 10$  cm.<sup>2</sup>

The respiration of this plant was taken as 0.17 per cent. That is the amount that the plant respire in the two hours immediately following a run, and was close to the interpolated value from the graph. After some hours in darkness it dropped to 0.04 per cent. Assuming complete absorption, no reflection, and the limit of photosynthesis at 720 mμ, the value varies from 8 quanta per molecule of carbon dioxide in the low light intensities to 12 in the high. Higher quantum yields could undoubtedly be obtained by longer acclimation of the plant to low light intensities. The loss by reflection and transmission was about 40 per cent. This would lower these values to 5 and 7.

The relative quantum yields are of more significance and are given in the following summary:

	NS 1	NS 2	ES 3	ES 4	P 3	P 4
Blue-violet . . . . .			..	47.5	53.5	54.3
Red-yellow .....	101	98	..	..	111.0	.....
Red .....		..	96.5	93.0	96.5	.. ..

The low values in the red may be due to the fact that photosynthesis stops before 720 m $\mu$  or to low absorption. The differences between the pine and the spruce seem larger than that which could be attributed to their difference in color. The very low values for the blue-violet must be due to an actual falling off of photosynthesis. The results with red-yellow also indicate this. Since white light was about 70 per cent. red-yellow, the efficiency of the remaining 30 per cent. must be low when 117 or 105 parts of white are necessary to equal 100 parts of red-yellow.

### Summary

1. A method for determining the relative quantum yields in photosynthesis in different portions of the spectrum has been described and the experimental errors determined.

2. Infra-red radiation of wave length longer than 1100 m $\mu$  is detrimental to photosynthesis.

3. With spruce the relative quantum yields in the wave lengths 720–630, 720–560, and 470–390 m $\mu$  are 9.5, 10, and 5; and with pine, 9.5, 11, and 5.

4. The actual quantum yield with pine is between 5 and 12 quanta per mole of carbon dioxide.

5. A survey of these results from a botanical aspect will be published from the Vermont Agricultural Experiment Station, where this work was carried on. These experiments are being continued.

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# EFFECT OF LIGHT ON THE BIOELECTRIC POTENTIALS OF ISOLATED *ELODEA* LEAVES

H. BENTLEY GLASS

(WITH NINE FIGURES)

## Introduction

The theory that the electric polarities of cells and tissues of living organisms are probably due to oxidation-reduction systems has been advanced by LUND and his associates (2). They have further suggested that such potentials constitute a mechanism of correlation between the cells or parts of the organism (2). The effect of light upon such systems seems therefore worthy of investigation. In 1892 OTTO HAAKE showed that green leaves increase their E.M.F.s in a positive direction when illuminated, and that other plant tissues, if chlorophyll-free, are not so affected (1). Since that time methods for the detection of electrical phenomena have been greatly improved, but the question of the effect of radiant energy upon bioelectric phenomena in plants has remained practically unexplored. A summary of the work done up to the present upon these problems is to be found in the paper by WALLER (7). In the present paper the results of a study of the responses made by single isolated leaves of *Elodea* are reported. This material was selected because of its simplicity of structure, each leaf having only two similar layers of large rectangular cells, and only one vein, the midrib; and because the absence of a cuticle facilitates the measurement of the potentials.

## Method

The potentials between the tips and basal ends of detached leaves were measured with a Compton quadrant electrometer, the electrical circuit being identical with the one described by LUND (3). Isoelectric saturated zinc sulphate electrodes were used, and contact with the leaves was made through glass contact cups and arms, containing tap water (fig. 1). The leaves were placed in a moist chamber covered by a hood to exclude the light. They were supported on the contact arms in an upright position, so that the basal end was connected to the grounded quadrants, and the apex to the opposite pair (fig. 1). Since diffusion through the capillary arms of the contacts was very slow, and the water in them was changed every day, there was no danger of zinc sulphate solution reaching the leaf and injuring it. The contact cups passed through holes in the base of the moist chamber. These openings were sealed with flexible rubber joints, permitting movement of the contacts from outside the chamber while maintaining its hermetic sealing. When the walls of the moist chamber were covered with wet

filter paper and the chamber was saturated with moisture, a leaf could be kept alive and in a normal state in it for many hours. The source of illumination was an electric globe with a linear filament, the beam being focused, after passing through a water chamber, by a microscope objective and ocular, then passing through a window into the moist chamber, and finally through the aperture of an iris diaphragm so as to fall upon the leaf

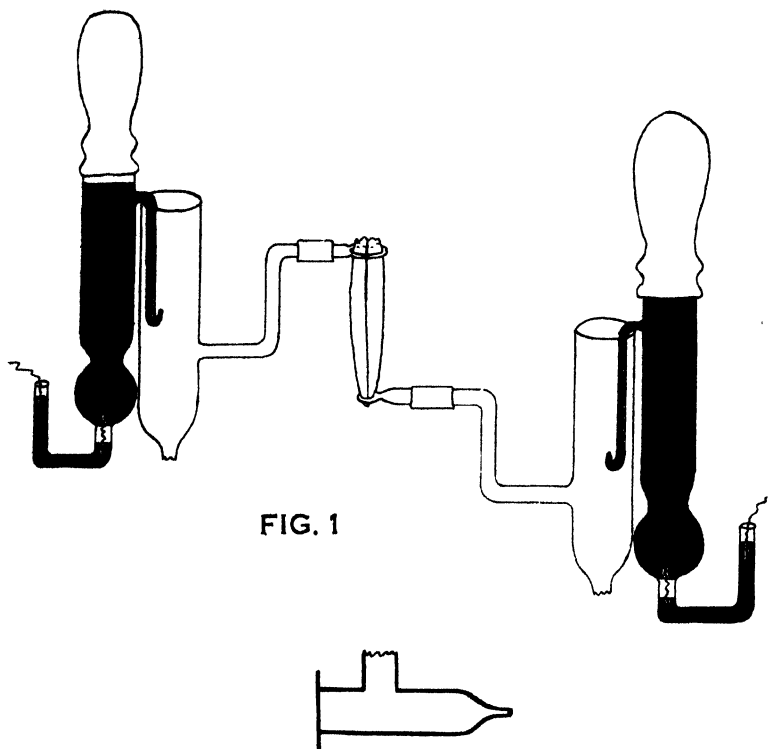


FIG. 1

FIG. 1A.

FIG. 1. Diagram of zinc sulphate electrodes, contact cups containing tap water, and contacts, with a leaf in position.

FIG. 1A. Special capillary contact for leading off at various points along surface of leaf.

in a small round spot 3 mm. in diameter, with an intensity of illumination of approximately 18,000 foot-candles. The light source was mounted upon a heavy base arranged to provide micro-manipulatory movement in three planes. All experiments were repeated at least three times, and more in the case of divergence. No two leaves react in precisely the same way, yet the general agreement is very good. Readings were plotted directly in graph form from the scale at 12-second intervals.

### Absence of effect of light on electric polarity of onion root

Preliminary readings were made on onion roots, 4 to 6 cm. in length. These remained intact upon the bulb in a perfectly normal state, and continued growth following the experiments. The usual large inherent potentials were observed, which fluctuated when the roots were mechanically stimulated; but absolutely no effect followed illumination. This result confirms the observations of HAAKE (1) and WALLER (7), and apparently definitely confirms the relation of the phenomena to be described in *Elodea* to the presence of the chloroplasts.

### Effect of light on electric polarity of *Elodea* leaf

The isolated leaves of *Elodea* were always dark-adapted, and allowed to remain in the moist chamber for a period varying from 40 minutes to 2 hours before illumination was begun. This was necessary in order that the effect of mechanical stimulation might subside, and that the potential might reach a stable level. This basal level of the electric polarity in a dark-adapted leaf was low, generally amounting to less than 10 millivolts between apex and base.

Experiments were conducted using both apical and basal leaves. By the former term is meant those leaves taken from a region within 2 inches of the growing tip, and of a light yellowish green color; by the latter term is meant one taken 10 or more inches from the tip, of a dark green color, and from a region where the whorls are spaced far apart.

Either apical or basal leaves, when placed in the moist chamber, showed that initially the apex was positive to the base. This E.M.F. was always found to be falling rapidly, as soon as observations could be made, and soon reached a level close to zero potential, either slightly above or below, depending upon the original condition of the leaf. If the hood was not

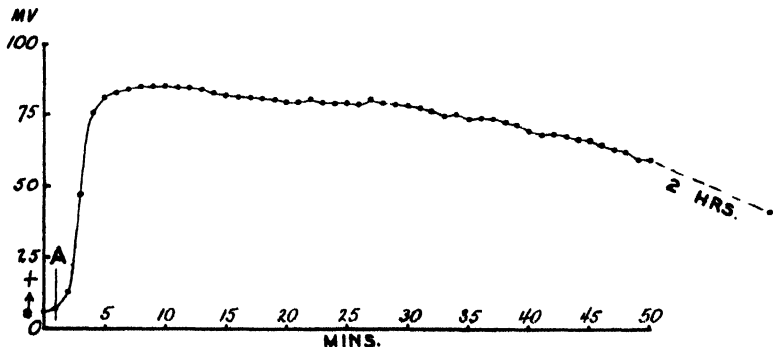


FIG. 2. Change of potential in an apical *Elodea* leaf under constant illumination of an apical spot; A, beginning of illumination.

placed over the leaf, it recovered to such a state that the apex again was positive to the base after a period of 1–2 hours, by an amount approximately equal to the originally observed potential. But when the leaf was placed in the dark, it found a new level of equilibrium, close to zero potential.

When a dark-adapted apical leaf of *Elodea* was illuminated by a round spot focused on the apex of the leaf, within a period of 5–10 minutes the potential between apex and base rose from nearly zero to a level between 75 and 100 millivolts (fig. 2). Under continuous illumination after the maximum response was attained, there was always found to be a slow

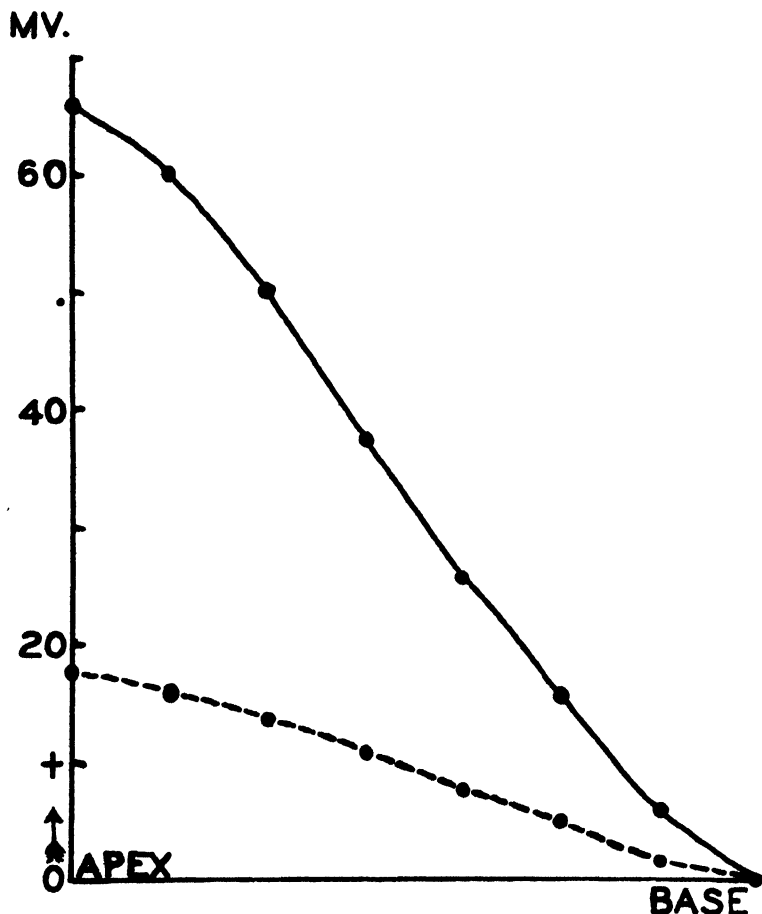


FIG. 3. Magnitude of E.M.F.s along an apical *Elodea* leaf, measured with respect to the base:

dotted line, when unilluminated;

solid line, when illuminated at an apical spot.

decrease in the magnitude of the potential. After several hours, it was only about half as great as at first. When more than one reading was made upon the same leaf, the successive ones were always of decreasing magnitude. WALLER (5, 6) has reported some evidence of "fatigue" in his experiments upon the effects of light on the potentials of green leaves. This seems to be confirmatory, therefore, but should not be regarded as conclusive evidence, since the observations were only incidental to the main purpose in view, and the conditions were therefore not sufficiently controlled to that end.

It has been stated that the apex is positive to the base before illumination. But often both apex and base are positive to the middle portion of the leaf (fig. 4). Yet the decrease of E.M.F.s along the leaf may be uniform, and the base then the point of lowest potential (fig. 3). Illumination of the apex causes its potential to be greatly increased over that of the base; and an increase of potential also occurs along the entire leaf, diminishing with distance from the point of illumination. The decrease of E.M.F.s along the leaf is uniform, and appears as practically a straight line in the graph (fig. 3). This occurred in all cases, regardless of the original orientation of E.M.F.s along the leaf. When the leaf was illuminated at the basal end, the base became markedly positive to the apex, and again the transmission of the effect of non-illuminated parts of the leaf was observed.

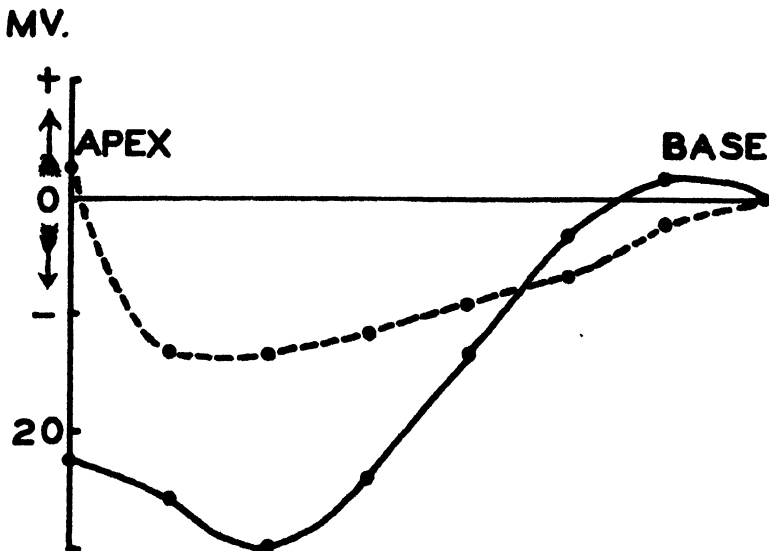


FIG. 4. Magnitude of E.M.F.s along an apical *Elodea* leaf, measured with respect to the base:

dotted line, when unilluminated;  
solid line, when illuminated at a basal spot.

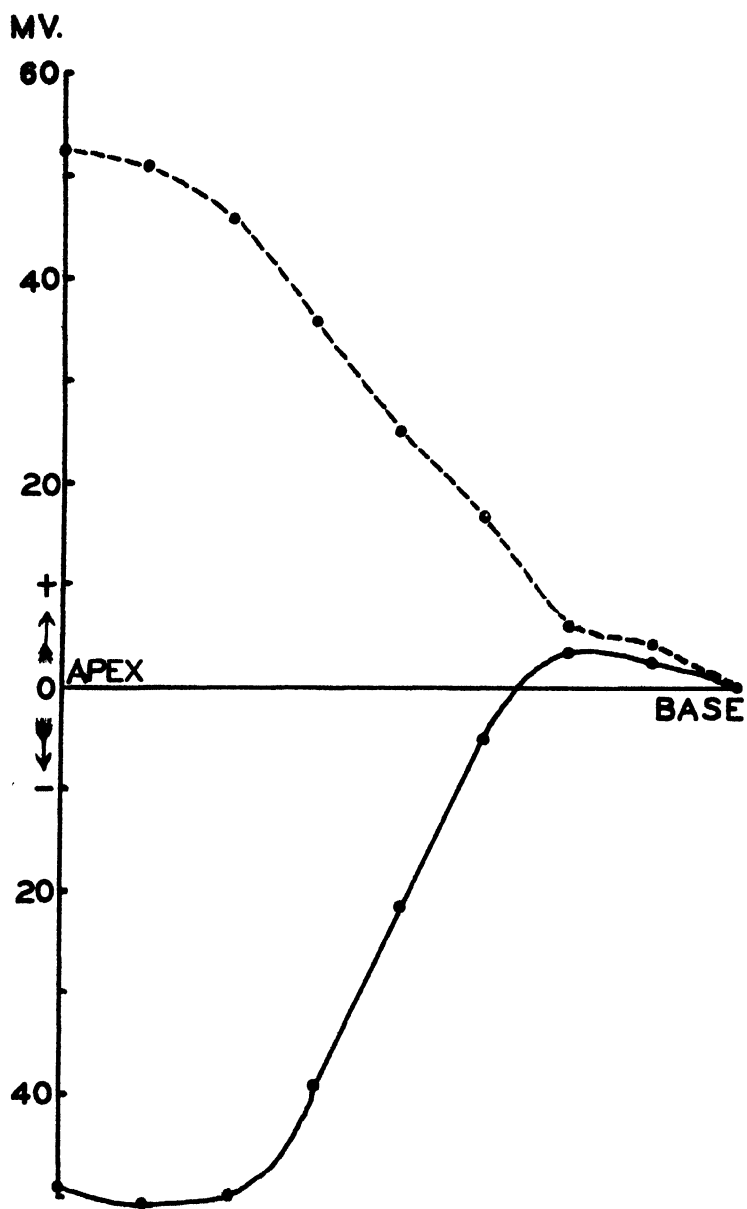


FIG. 5. Same as in figure 4.

This too occurred regardless of the original orientation of the E.M.F.s (figs. 4, 5). The solid lines on both these figures show, however, that there is a short region close to the tip of the leaf where the original orientation is preserved. It is further to be observed in these two figures that the E.M.F. is at a maximum at the point of application of the light, 2 to 4 mm. from the base. Again, when a point outside the positions of the contacts was illuminated, the region between the contacts being maintained in darkness, transmission of the effect of light to the region between the contacts was observed in the same manner. In plotting the magnitudes of the E.M.F.s along the leaf, the contact at the base was connected with the grounded quadrants, as before; while a new capillary tip contact was made which could be moved up or down at will, and also moved back from the leaf surface, thus avoiding stimulation of the leaf by motion of the contact along it; actual contact was made only through the drop of tap water at the tip (fig. 1A). Figures 4 and 5 show that although the base of the leaf was illuminated and the apex was maintained in darkness, yet apparently the potential at the apex has been greatly altered while that at the base has been maintained constant. It must be emphasized that this effect is only apparent, owing to the conditions of the experimental technique. It is due to the fact that, as in the experiment graphed in figure 3, the base of the leaf was connected with the grounded quadrants. What actually occurs is that, as all other evidence shows, the potential of the base is greatly increased when it is illuminated, while that of the apex, remaining in darkness, is relatively constant.

Apical leaves react to illumination in the typical manner shown in figure 6. Curve I represents the change of potential under constant illumination of a spot at the apex of the leaf; curve II represents the same change when a spot in the center of the leaf is illuminated; and curve III similarly for a spot at the base. The time at which the light was turned on is marked by the vertical line at *A*, and the time at which it was turned off by the arrow at *B* upon each curve. In each case the response is rapid, reaching the maximum in 7 to 10 minutes. As soon as the illumination ceases, the potentials promptly drop. The response when the apex is illuminated is always positive, *i.e.*, the potential of the apex as compared with that of the base is increased. It is large in magnitude. When the central portion is illuminated, the response is always positive but is much less in magnitude; when the base is stimulated the response is again large, but is in a negative direction, *i.e.*, the potential of the base with respect to that of the apex is increased. This response is sometimes even larger than the positive response when the apex is illuminated. It was also noted that in the second of the three cases the response varied considerably, being sometimes large and sometimes small. Twice positive responses were obtained, although of

slight magnitude, when the basal end was illuminated. These exceptions to the general case were probably due to poor material.

Basal leaves were also similarly illuminated (fig. 7). The magnitude of the responses, other conditions being the same, appear to be smaller than the responses in apical leaves.

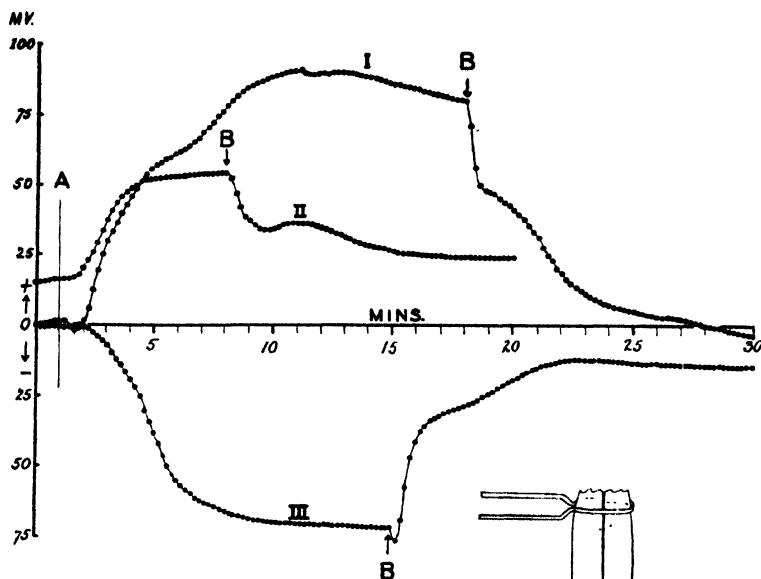


FIG. 6

FIG. 6A

Fig. 6. Change of potential in an apical *Elodea* leaf under illumination:

I, at apical spot (see fig. 6A);

II, at spot in center of leaf;

III, at basal spot.

A marks the instant at which illumination began; B the one at which, in each case, it ceased.

Fig. 6A. Diagram of an *Elodea* leaf in position on contacts. The circles mark various positions illuminated.

Examination of the individual curves (figs. 6, 7) shows certain constant features. There is a slight lag of about a minute after the illumination begins before any response can be observed. The initial response is sometimes a change in the opposite direction to that in which the succeeding main response occurs. The steady decrease after maximum response is attained has already been mentioned. When illumination ceases, there is a

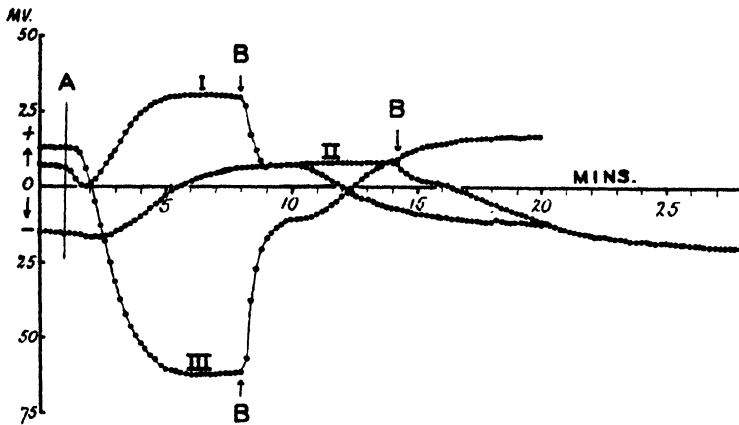


FIG. 7. Change of potential in basal *Elodea* leaf; notation as in figure 6.

sharp drop immediately, then a slowing down of the rate of decrease of potential, causing a flattening of the curve which may sometimes even take the form of a slight rise; this is then followed by a further rapid fall to approximately the original level of the potential between apex and base. There is no "over-shooting," that is, the potential reaches its final level slowly, not passing by it and then returning to it. In about 20 per cent. of the cases the double character of the descending curve cannot be discerned, perhaps because it is too slight.

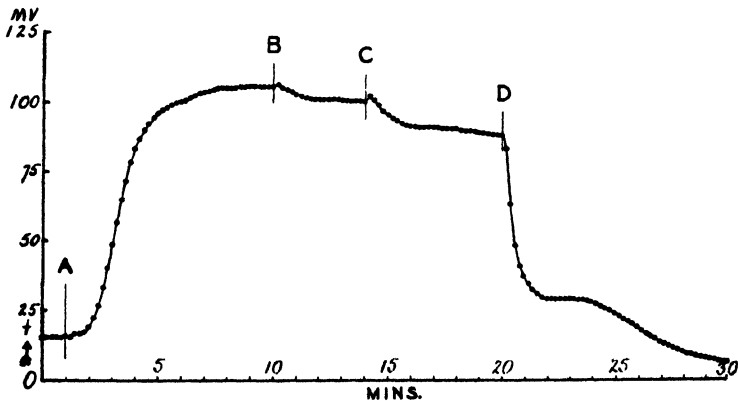


FIG. 8. Effect of decreasing intensity of illumination upon the potential of an apical *Elodea* leaf illuminated at apex:

- A, illumination began; intensity 18,000 foot-candles;
- B, intensity decreased to 9000 foot-candles;
- C, intensity decreased to 1700 foot-candles;
- D, illumination ended.

Preliminary experiments were also conducted upon the effect of varying the intensity of the incident light. This was done by means of a resistance inserted into the lighting circuit, capable of cutting down the intensity of the illumination of the spot upon the leaf from 18,000 to approximately 1700 foot-candles. It was found that when the intensity of the light was first applied at this maximum and then diminished, a corresponding diminution of potential was to be observed (fig. 8); but when the intensity of the light was first applied at 1700 foot-candles, the response observed was the maximum, further increase of the intensity failing to raise the potential (fig. 9). Evidently the intensity of the light is not the only limiting factor concerned here. Further work is being planned to determine this point fully.

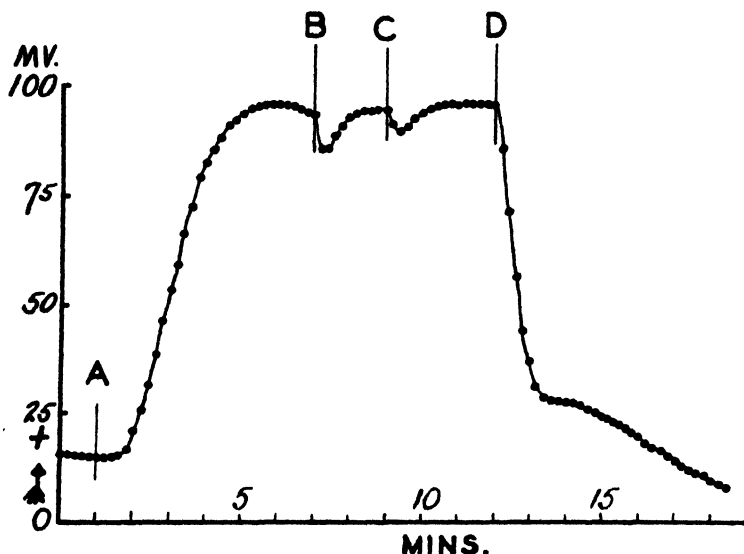


FIG. 9. Effect of increasing intensity of illumination upon the potential of an apical *Elodea* leaf illuminated at apex:

- A, illumination began; intensity 1700 foot-candles;
- B, intensity increased to 9000 foot-candles;
- C, intensity increased to 18,000 foot-candles;
- D, illumination ended.

### Discussion

WALLER (8) reported potentials from many plants. The highest potential he observed amounted to only 15 millivolts under illumination. SHEARD (4) reported 0.3 volt in sunflower leaves under illumination; even this high potential, in relation to the area of leaf surface, is small in comparison with the potentials reported in the present paper for *Elodea*. The leaf of the

latter measures only 10–12 mm. in length and 3–5 mm. in width, yet potentials up to 100 millivolts have been observed under illumination between its apex and base. It should be evident, both from the size of the potentials observed and from their great constancy of behavior, that we are here concerned with something connected with the fundamental energy processes of the organism. It can hardly be believed that any expenditure of electrical energy by a plant in which the potential of a single leaf may vary by as much as 100 millivolts can be either insignificant or of secondary importance.

The relation of photosynthesis to these phenomena should prove of decided interest. The peculiarities of the curves found should throw further light on the question, particularly as to the chemical and physical processes involved, which give rise to them.

Transmission of the effect of illumination to non-illuminated regions is obvious. Illumination of a point on the leaf outside the region between the contacts has also been found to change the magnitudes of the E.M.F.s between the contacts. Figure 3 is particularly clear in showing such transmission. It should be observed that, since the decrement of E.M.F. along the leaf is practically a straight line, this means that the effect of illumination is felt not only at non-illuminated regions, but that the effect extends throughout the leaf, regardless of the distance from the point illuminated. This makes it very probable that the effect of light upon the potential of the leaf is not a direct photoelectric effect, but that it reacts first upon the chloroplasts, which in some way set up changes in the state of the leaf, giving rise in turn to the electric phenomena.

### Summary

1. Apical and basal leaves of *Elodea* respond to illumination of the apex of the leaf by a large increase of the potential of the apex with respect to that of the base. The decrease in the magnitudes of the E.M.F.s along the leaf from apex to base in such a case is uniform.
2. Apical and basal leaves illuminated in the middle show a positive response, but to a less extent than when illuminated at the tip.
3. Apical and basal leaves illuminated at the base show a reversal of the electrical polarity found in the two preceding cases. The base becomes positive to the apex.

The writer wishes to express his deep appreciation of the material assistance rendered him in the present survey, both in the matter of apparatus and advice, by Dr. E. J. LUND.

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# RELATION OF HYDROPHILIC COLLOIDS TO HARDINESS IN CABBAGE, BRUSSELS SPROUTS, AND ALFALFA PLANTS AS SHOWN BY THE DYE ADSORPTION TEST

STUART DUNN

(WITH THREE FIGURES).

## Introduction

If we assume that hydrophilic colloids are largely responsible for hardness in plants, then the amount of dye removed from solution by plant tissues suitably prepared will bear a direct relation to the degree of hardness exhibited. In previous work by the writer (1, 2), in which anilin dyes and more particularly malachite green were used in a study of hardness in the apple, somewhat conflicting results were obtained, traceable in part to the material and in part to the mode of sampling. Further experiments were thought desirable, and the present paper describes the results secured with cabbage, brussels sprouts, and alfalfa, which could be grown in pots in a controlled environment.

## Materials and methods

Except in the case of alfalfa, where field-grown plants were used in some instances, all the plants used were grown in the greenhouse. For some of the hardening experiments, the potted plants were stored at 3° C. for various lengths of time in a lighted electric refrigerator, while other sets of plants for purposes of control were stored at room temperature in a specially constructed artificial light chamber and thus were grown under similar conditions except temperature.

Freshly macerated tissue was used in nearly all tests as it most closely resembles the colloidal conditions of the plant cell. The grinding was done by pressing the material against a rotary, motor-driven wire brush. Brussels sprouts were used in a nearly mature state, and their pith provided a perfectly uniform tissue obtained by paring away the outer parts.

After the tissue was ground, 1-gram samples of it were at once weighed into centrifuge tubes on a torsion balance sensitive to 5 mg. To each sample was added 25 cc. of the dilute dye solution (in the case of malachite green usually at a strength of 0.1 gm. per liter), and the tubes shaken vigorously for several minutes, centrifuged, and the supernatant liquid poured off into a test-tube for clearing. A considerable amount of the plant material remained in solution or suspension in the dye solution, even after centrifuging, which interfered somewhat with obtaining an accurate colorimetric reading. It was found that most of this could be coagulated by heating

in a boiling hot-water bath for three minutes. Tests on pure dye solution showed this treatment to have no effect on the color intensity of the dye. After cooling, the sample was filtered through an asbestos mat in a Gooch crucible. A portion of the solution was first run through and thrown away, to avoid the effect of adsorption of the dye by the asbestos. After filtration, the sample was tested for the amount of dye remaining in solution in a Leitz bicolorimeter, following the conventional procedure (1). In most cases it was possible to obtain a good match of colors without resorting to a second wedge containing a distilled-water extract of the plant tissue.

For check on the actual hardiness of different groups of plants, some of them were frozen at  $-5^{\circ}$  C. in a cold chamber in the cold storage plant of the Horticultural Department. In hardening off large plants such as mature brussels sprouts, which were too large to be put into the refrigerator, they were placed in another chamber of the cold storage plant at a temperature of  $0^{\circ}$  C. Since HARVEY (3) has shown that hardening is probably a cold shock response, and that exposure of plants to short periods of cold daily is very effective, in most cases the plants which were being hardened were placed in the cold chambers daily from 5 to 9 P. M.

Alfalfa plants presented some peculiar difficulties. Freezing tests showed that the tops were all killed in most treatments so that observations and tests had to be confined to the roots. Not being readily visible, like the tops, they must be removed from the soil and washed for accurate examination, which damages them for observation of injury at subsequent intervals. Their tough and woody nature renders freezing injury difficult to detect.

### Experimentation

INVESTIGATIONS ON CABBAGE.—To gain comprehensive information as to the effect of different lengths of time of hardening on the colloid content of cabbage plants, a large number of plants were hardened for various periods from 1 day up to 12 days. HARVEY has shown that 5 days of hardening is sufficient to show marked differences between hardened and unhardened plants. Similar lots of plants were also submitted to a freezing test as a check on their cold resistance. The dye adsorption results are given in table I. The figures represent the percentage of dye left in solution after adsorption, and thus the lower values indicate the greater hardiness. Individual plants were ground separately, all parts above ground being used.

It will be seen that in general the average adsorption increases up to the 4- and 5-day periods, but beyond that there is somewhat of a decrease, especially in the group hardened for 12 days, which became rather unhealthy in appearance. In all cases the average adsorption was greater in the hardened plants than in the unhardened. It will be noted, however,

TABLE I

ADSORPTION OF MALACHITE GREEN BY MACERATED FRESH CABBAGE

TREATMENT	PERCENTAGE DYE REMAINING IN SOLUTION		
	MEAN*	MAXIMUM*	MINIMUM*
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Hardened 1 day	32.4	46	21
Unhardened	40.4	59	31
Hardened 2 days	36.8	65	20
Hardened 3 days	33.1	55	23
Unhardened	39.3	57	25
Hardened 4 days	26.9	49	19
Hardened 5 days	29.8	45	20
Hardened 6 days	33.7	53	28
Unhardened	38.3	51	24
Hardened 8 days	37.4	50	24
Hardened 12 days	37.5	57	23

\* From duplicate tests on 8 plants.

that the individual values for the different plants show great variability, from extremely low readings of around 20 up to high ones above 60. Each group has certain of these variations, even the unhardened lots. This naturally leads to the question of how the similar groups of plants behaved in simultaneous freezing tests. In most cases the results agreed in general with those of the dye adsorption tests in table I. Here also, however, wide

FIG. 1. Cabbage plants hardened for 3 days, then frozen for 24 hours at  $-5^{\circ}\text{C}$ .

variation was found in the ability to resist freezing on the part of individual plants in all of the groups. Those affected most were completely wilted after removal for a short time from the freezing chamber, while others appeared almost unharmed. Various degrees of resistance on the part of some of these plants may be seen from the accompanying photographs. Figure 1 shows cabbage plants hardened for 3 days and then frozen at  $-5^{\circ}\text{C}$ . for 24 hours. Considerable variation may be seen, from one almost totally killed to one harmed only slightly. Even more variation is shown in the group in figure 2, which was hardened for 5 days before freezing. It is apparent that the group hardened for 3 days averaged less hardy than the group hardened for 5 days. Nevertheless the individuals of both groups show extreme variations, and this is probably the reason for some, if not most, of the variations found in the dye adsorption tests.



Fig. 2. Cabbage plants hardened for 5 days, then frozen for 24 hours at  $-5^{\circ}\text{C}$ .

It was observed also that different parts of the same plant, or different tissues, were differently affected, as can be noted from figures 1 and 2. In most cases the leaf blade was injured more than the petiole or remainder of the plant. This led to tests in which the leaf blades were not included, and the results of some of these are given in table II. In all cases there is, on the average, a greater adsorption on the part of the hardened plants, although there is still some variation by individual plants within the groups. It will be noted that in the groups of youngest plants, 6 weeks old, the difference in adsorption by hardened and unhardened plants is not so great as that of older plants. This is in accord with observations on the results of freezing tests. In general the older plants withstood freezing better than

TABLE II

ADSORPTION OF MALACHITE GREEN BY HARDENED AND UNHARDENED CABBAGE STEMS AND PETIOLES OF PLANTS VARYING IN AGE

AGE OF PLANTS	TREATMENT	PERCENTAGE DYE REMAINING IN SOLUTION		
		MEAN*	MAXIMUM*	MINIMUM*
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Six weeks old	Hardened 4 days	54.8	58	52
	Unhardened	59.3	62	54
	Hardened 6 days	55.4	58	52
	Unhardened	59.6	65	54
Eight weeks old	Hardened 3 days	64.0	68	58
	Unhardened	68.4	76	61
	Hardened 4 days	60.9	66	53
	Unhardened	67.2	70	65
	Hardened 5 days	65.2	75	57
	Unhardened	69.6	77	64
Ten weeks old	Hardened 2 days	64.1	66	61
	Unhardened	76.0	79	73
	Hardened 3 days	73.7	80	65
	Unhardened	76.1	85	75
	Hardened 4 days	58.9	68	50
	Unhardened	75.0	78	68
	Hardened 5 days	54.0	57	50
	Unhardened	74.7	78	66

\* From duplicate tests on 5 plants.

the younger ones. In the case of the petioles and stems of the cabbage plant, however, it is very difficult to determine by inspection the exact effect of freezing, since the larger amount of mechanical or supporting tissue renders the effect of the collapse of frozen cells less visible than that of leaf tissue. This greater proportion of supporting tissue may also be responsible for the smaller amount of adsorption where these parts of the plant were tested alone, than where the leaves were included (table I). This is even more strikingly noticeable in table III.

In view of the marked variations in the survival of individual plants within a group whose previous hardening periods or other previous treatment has been the same, and the possibility that this may also account for some of the variations encountered in the dye adsorption figures, an effort was made to test this by comparing plants unharmed by freezing with those injured. It should be noted here also that often there were marked differences in different parts of the same plant. The lower leaves frequently were killed and flabby and the upper leaves were not; or a part of a single leaf might be affected and another part not. The results given in table III are for groups of plants of somewhat different ages, but the

TABLE III

ADSORPTION OF MALACHITE GREEN BY HARDY AND NON-HARDY CABBAGE PLANTS DIFFERENTIATED BY FREEZING TEST

AGE OF PLANTS	TREATMENT AND CONDITION	PERCENTAGE DYE REMAINING IN SOLUTION					
		MEAN*		MAXIMUM*		MINIMUM*	
		LEAVES	STEMS	LEAVES	STEMS	LEAVES	STEMS
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
One month old	Hardy 3 days	30.0	59.0	39	61	24	55
	Non-hardy	33.5	67.7	35	69	31	66
	Hardy 7 days	32.5	63.0	36	66	25	60
	Non-hardy	38.0	74.2	40	77	35	71
Two months old	Hardy 4 days	35.5	57.4	38	65	32	52
	Non-hardy	44.4	65.9	49	68	40	62
	Hardy 6 days	33.3	57.1	36	60	31	53
	Non-hardy	43.6	64.1	46	68	40	59
Three months old	Hardy 2 days	38.4		45		31	
	Non-hardy	53.5		60		51	
	Hardy 5 days	31.3		35		27	
	Non-hardy	52.4		58		50	

\* From duplicate tests on 5 plants.

outstanding fact is that the values show a uniformly greater adsorption by the hardy plant tissue than by the corresponding tissues of the non-hardy group. This is particularly true of the leaves. In the case of stem and petiole tissue, as has been noted before, it is often extremely difficult by examination to detect differences in these parts of the plant. This may explain partly some of the wider variations for these tissues given in the columns labeled stems. The results on the oldest plants, where only leaves were tested, confirm this more amply, and show that when it can be proved that a plant or plant part or tissue is hardy, then there will be a greater adsorption of dye by macerated samples of that tissue in comparison with non-hardy tissue.

An objection to this method of testing the plant tissue for adsorption after freezing the plant might be raised on the ground that the non-hardy plants which were so treated might have their colloidal properties altered by the effect of the freezing alone. An effort was made to determine this point by testing leaves of cabbage, from the same plant, cut in half lengthwise through the midrib. One group of halves was frozen and ground separately, while the unfrozen halves were also ground separately and tested for adsorption. Similar tests were made on whole cabbage plants halved lengthwise. In all cases the differences were slight and negligible.

This shows that the freezing is not a factor in producing the adsorptive differences noted in hardy and non-hardy plants.

INVESTIGATIONS ON BRUSSELS SPROUTS.—Large brussels sprouts plants were also tested, and the pith tissue of these plants was used because it is perfectly uniform, containing only one type of cell, with no fibrous elements not easily macerated. As in the cabbage plants, the average adsorption was greatest in those exposed to the hardening temperature for about 5 days. Here also, as in tests on cabbage plants, some attempt was made to select only those which definitely withstood freezing at  $-5^{\circ}\text{C}$ . for 12 hours or more as being hardy for dye adsorption tests. In the case of the pith tissue of these plants, however, it was much more difficult to detect differences in appearance of those injured. A water-soaked, darkened appearance was about all that could readily be seen. Frequently those plants having a hardy-appearing pith would have tender leaves and sometimes the reverse was true. Figure 3 gives an idea of the differences encountered after plants which had been treated similarly were frozen. These had



FIG. 3. Brussels sprouts hardened for 5 days, then frozen for 12 hours at  $-5^{\circ}\text{C}$ .

both been hardened for 5 days before being frozen for 12 hours at  $-5^{\circ}$  C. Aside from these difficulties the results confirmed those upon cabbage, and for the sake of brevity the tabular data are omitted.

**SPECIFIC ADSORPTION.**—An attempt was made to narrow the field still further, from hydrophilic colloids in general, as indicated by malachite green, to some one chemical constituent or group of constituents. This was tried by the action of other dyes, some of which are more or less specific in their adsorption by certain groups of compounds. One of these was ruthenium red, which is a standard stain for pectic substances, and two others, Ponceau 3 R and light green S F, which are partially cytoplasmic stains and non-toxic. Two others were safranin and haematoxylin. The former stains lignin, cellulose, suberin, and protoplasm in histological preparations unless it is washed out, in which case it primarily stains lignin and suberin. Sometimes it is used as a precipitating agent for proteins. Haematoxylin stains cellulose walls only. Malachite green was also used as a check on the hardness qualities.

Since these dyes were diluted for adsorption tests to about one-twentieth of the concentration used in histological work, microscopic studies were made with them at these dilutions, using cross-sections of young cabbage petiole and fresh *Elodea* leaves. The observations indicated that with safranin there was uniform penetration of most cells and accumulation in the vascular or heavily lignified cells and in the outer cutinized epidermis. Haematoxylin accumulated mainly in walls of parenchyma and moderately in the bast fibers and collenchyma of the cabbage. Light green S F and Ponceau 3 R penetrated the tissue fairly uniformly but plasmolyzed the cells slightly. No part of the protoplast was penetrated and there was no particular difference in color in any tissue. Apparently scarcely any of the dye was removed from solution. Malachite green penetrated the protoplasts of many cells of *Elodea* fairly uniformly, especially the chloroplasts and other plastids, but was not noticeable in the walls. In cabbage sections there was a somewhat noticeable accumulation in the cells rich in protoplasm near the outer edge, and in the vascular tissue.

A large number of cabbage and brussels sprouts plants were passed through a freezing test, and an outstanding plant showing marked hardness and similarly one which was non-hardy were chosen from each. Each of the dyes was tested for adsorption on several samples from the same plant, using the usual colorimetric technique. It was found that with safranin there was a greater adsorption by the non-hardy samples. This would indicate that perhaps in the hardening process there is a conversion of lignin or allied materials and proteins into something else, perhaps some protoplasmic or cell sap constituents. With light green S F there was very slight adsorption, especially in the brussels sprouts, and with Ponceau 3 R

none at all. This agrees well with the observation on microscope sections already noted; and since there was the usual greater adsorption of malachite green by the hardy tissue, this would indicate that some of the protoplasmic constituents are probably primarily responsible for hardness. Haematoxylin gave slight differences, indicating that the cellulose wall constituents are of slight importance in hardness. A somewhat greater adsorption of ruthenium red by the hardy tissue, especially in brussels sprouts, shows that pectic substances may have a part in the colloidal protection against low temperature.

INVESTIGATIONS ON ALFALFA.—Seed of the same two varieties of alfalfa as those used by STEINMETZ (4) in his investigations was obtained from the same source. They were Kansas Common, a rather tender variety, and Grimm, a hardy variety. The seed was planted in outdoor plots in the spring. During the following winter and spring, samples of the roots were dug up in February, March, and April and tested for adsorption in the macerated fresh condition, and also dried and ground to a fine powder. It was found that the fresh samples of February and April gave tests correlating with the hardness of the two varieties, while the March samples did not. The dried samples showed very little correlation. Even when the percentage of dry weight was taken into consideration, the differences were not sufficient to make that a factor. Presumably the heating in some way alters the colloidal substances of the tissue, and tests on fresh tissue may be regarded as more indicative of hardness.

Other tests were run on hardened and unhardened potted plants. Efforts were first turned toward preliminary tests on the killing point of roots by freezing, using the water-soaked appearance and exudation of sap from a cut end mentioned by STEINMETZ as criteria of injury. All plants were old, with large roots, having been grown in large pots in the greenhouse for a long time. The Grimm and Kansas Common were used, and also a variety called Cossack, from Texas, supposedly tender. The time for killing roots removed from soil varied between 1 hour and 2.5 hours at  $-4^{\circ}$  C.

This work was attended with the difficulties mentioned under Methods, but in several instances differences were noticeable a short time after thawing, and results on a few of the many adsorption tests run are given in table IV. The first two groups, those unhardened and those hardened 11 days, show a greater adsorption by the hardy tissue, as anticipated from previous work. The other 9-day plants show conflicting results.

In a few other tests, from time to time, discrepancies of this nature were encountered, which led to some doubt as to the advisability of placing very great confidence in the water-soaked appearance of the root and other similar criteria as evidence of injury. Furthermore, WEIMER (5) has

TABLE IV

ADSORPTION OF MALACHITE GREEN BY MACERATED GRIMM ALFALFA ROOTS; INJURY DETERMINED BY INSPECTION SHORTLY AFTER FREEZING

TREATMENT	APPEARANCE	PERCENTAGE DYE REMAINING IN SOLUTION		
		MEAN*	MAXIMUM*	MINIMUM*
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Unhardened	Not injured	19.6	24	18
	Injured	26.2	28	25
Hardened 11 days	Not injured	20.6	21	20
	Injured	36.2	47	29
Hardened 9 days	Not injured	31.7	38	26
	Injured	24.0	25	23

\* From 5 samples.

shown that different regions or tissues, or even separate groups of cells, in the same alfalfa root may show differences in survival in freezing tests. This led to final attempts to determine more accurately the relation between dye adsorption and frost survival, by freezing hardened and unhardened roots and then placing them in wet sand to allow those surviving to bud out. Those showing differences were then selected for dye tests. One group of all three varieties was tested in this way after hardening for 8 days, and after a few days the hardened plants in most instances budded out more vigorously and a few of the unhardened ones were entirely killed. The differences in the Texas Cossack were very slight, and this is also true of the dye test on this variety. Results appear in table V.

TABLE V

ADSORPTION OF MALACHITE GREEN BY MACERATED ALFALFA ROOTS; INJURY AFTER FREEZING DETERMINED BY BUDDING OF ROOTS IN MOIST SAND

VARIETY	CONDITION	PERCENTAGE DYE REMAINING IN SOLUTION		
		MEAN*	MAXIMUM*	MINIMUM*
		<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Grimm .....	Hardy	26.7	30	23
	Non-hardy	48.0	52	41
Kansas Common	Hardy	16.7	19	14
	Non-hardy	32.5	40	24
Texas Cossack	Hardy	23.5	30	19
	Non-hardy	18.5	23	16

\* From 4 samples.

In this case also, it seems evident that hardiness, as demonstrated by survival of or resistance to freezing, is correlated with dye adsorption and therefore with hydrophilic colloid content; although even here it is exceedingly difficult to be sure of actual differences in survival.

As mentioned before, previous work on apple-twig tissue showed wide variations and disagreements in hardiness and dye tests. In the light of the results presented here, most of the seeming lack of correlation there was probably due to a lack of definite and accurate knowledge of the actual hardiness, not only of the individual tree but of the individual twigs used as samples for the dye adsorption tests. It is true that parallel freezing tests were made at the same time as the dye tests, but they were on other twigs, and from the preceding evidence it seems probable that at least some of the differences were due to this.

Another factor which may be of importance in this connection is that in ligneous plants of this type there is present in the twigs, and consequently in the samples tested, a large amount of tissue extraneous or foreign to the tissues actually involved in hardiness. That is, even though the colloid content of the living active cells concerned in the hardiness and vigor of the tree may be the main factor in hardiness, the percentage of these cells is so small as compared with other kinds of tissue that their effect is masked. This factor has already been mentioned in connection with the relation between the fibrous elements and visibility of killing effects in cabbage petioles and stems and alfalfa roots.

### Summary

1. Previous investigations using the adsorption of the dye, malachite green, as a measure of the content of hydrophilic colloids in relation to hardiness in apple-twig tissue had been somewhat conflicting. Investigations were made on small succulent potted plants because their environment and growth conditions could be more readily controlled.

2. Dye adsorption tests were performed on cabbage, brussels sprouts, and alfalfa plants macerated in the fresh condition, and on dried tissue, to determine colloid content and to determine its relation to hardiness as shown by freezing tests on plants by artificial refrigeration.

3. Random tests on a large number of cabbage plants hardened for varying lengths of time showed that, on the average, hardiness as shown by dye adsorption increased over the unhardened plants up to 5 days' hardening. There were, however, marked extremes in values in both hardened and unhardened individuals. This, in general, agreed with results on parallel freezing tests where also extremes were met.

4. Tests on cabbage and brussels sprouts, in which actual survival ability or frost resistance of the individual plants was first tested by freezing,

showed that where this could be accurately determined, usually there was a greater amount of dye adsorbed from solution by such tissue, and the hydrophilic colloid content thus measured is probably in considerable degree responsible for hardness.

5. Efforts were made to narrow the field from hydrophilic colloids in general to some specific group of colloids. Adsorption tests by different dyes which vary somewhat in their adsorptive action showed that: (1) pectic substances may be partially responsible for cold resistance; (2) lignin and allied substances and perhaps proteins are apparently converted into other compounds in the hardening process; and (3) protoplasmic constituents rather than those of the cell wall are probably of primary importance in hardness of the plant cell to cold.

6. Work on alfalfa roots showed that here, too, dye adsorption was greater in the hardier plant, although results of freezing tests are more difficult to interpret because of the tough, woody nature of the tissue.

7. It is suggested that the conflicting results obtained in some of the previous work on apple-twig tissue may have been due partly to the lack of accurate knowledge of the cold resistance of the twigs actually tested, and partly to the fact that the vital tissue concerned in hardness is a very small part of the total bulk of the twig, so that the colloidal effect of such tissue would be masked by other inactive parts.

The writer wishes to express his sincere appreciation to Dr. R. B. HARVEY of the University of Minnesota and to Dr. O. BUTLER of the University of New Hampshire, whose kindly criticisms and advice have aided these investigations.

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# STIMULATIVE EFFECTS OF X-RAYS ON PLANT GROWTH<sup>1</sup>

CHARLES A. SHULL AND JOHN W. MITCHELL

(WITH FOUR FIGURES)

## Introduction

During the period since the discovery of x-rays by RÖNTGEN in 1895, a vast amount of work has been done in which these radiations have been used for clinical diagnosis and therapy. The practical applications of x-rays in medicine and surgery make it necessary to know the effect which x-rays produce upon the living organism. Many investigators have suggested on the basis of general observations that small doses of x-rays may stimulate cellular activity and growth, but convincing proof of such action has been wanting. In more recent years such claims have been discounted in favor of the belief that x-rays are always more or less destructive in action, and tend to retard growth.

It is not the purpose of this preliminary report to survey the literature dealing with the effects of x-ray treatments upon plants. It has been found that every part of the plant body can be profoundly modified by appropriate treatments. Cytological and histological examination of treated cells and tissues reveals striking changes in the organization of the protoplasm and of organs derived from the treated meristems. Most frequently the results described are of a destructive nature. The protoplasm is partially disorganized; chromosomes are vacuolated or fragmented; the cell division mechanism functions imperfectly, showing unequal distribution of chromosomes, non-disjunctions, translocation of pieces of chromosomes from one to some other non-homologous chromosome, etc. Gene changes may be produced, often injurious in character, with resulting lethal effects and tendency to sterility. The results obtained by MCKAY and GOODSPEED (5) on cotton are typical. Many mutations have been induced in maize and barley (7, 8), and tobacco (1), but it has been questioned whether there are any progressive evolutionary changes induced by x-ray treatments.

All vegetative parts are subject to injury by x-rays. Root tips may become bulbous and swollen, with tumor-like enlargements in which giant cells may occur. Stems become fasciated under strong treatments. Leaves are injured readily; they become asymmetric and crumpled in appearance, develop deep sinuosities, and often show irregular development of chlorophyll. The sunflower shows these injuries in typical fashion, the leaves becoming pocked and marked as though they were suffering from a mosaic

<sup>1</sup> This investigation was aided in part by a grant to the University of Chicago from the Rockefeller Foundation.

disease. Even the flowers of plants rayed in seed or seedling stages may show fasciation or various teratological modifications. Some of these have been described for the sunflower and tomato by JOHNSON (2, 3).

On the other hand, one can find a dozen or more claims in the literature that x-rays in small doses are stimulative. In some cases increased yields have been claimed for crops grown from x-rayed material. Such claims have been reinvestigated in some cases, and the stimulative effects denied. JOHNSON (4), for instance, has not been able to substantiate such claims made for the potato. However, some increase of yield has been reported for x-rayed potatoes at the New Jersey Agricultural Experiment Station. PATTERSON and MULLER (6) have found that induced point mutations in *Drosophila* (presumably caused by chemical changes in the genes) may cause increased vigor in some cases. They argue in favor of the possibility of progressive x-ray mutations with endless eventual potentialities.

As a result of our experiences with the use of x-rays on plants it is believed that stimulative effects may be consistently obtained if appropriate conditions are employed. Possibly these stimulative phenomena have not been regularly detected in the past because the intensity of the radiations have been too great, or possibly because the x-ray beam contained too large a proportion of long wave-length radiation. Deleterious effects are consistently obtained in our work when unfiltered radiations are used, and we believe that these harmful effects mask the stimulation that occurs when the beam is properly filtered. Filtration of the radiation, of course, affects the wave-length constitution of an x-ray beam profoundly. It not only reduces the intensity of each wave length throughout the x-ray spectrum, but also changes the relative proportion of the energy supplied by each wave length throughout the spectrum. The shorter radiations suffer much less absorption than the longer radiations; and for practical purposes the longest x-rays are so strongly absorbed by aluminum or copper filters that filtration through such metal plates practically removes them from the beam.

Since filtration affects both the intensity and relative composition of the beam, and since we have not yet differentiated these effects in our work, we are not in position to discuss the nature of the x-ray action. Until further experiments are done we cannot say whether the stimulating effects that are obtained when the beam is filtered are due to the fact that harmful long wave-length rays are removed, or whether they simply indicate that stimulation follows low intensity irradiation, regardless of wave length, and is masked by injury if the intensity is greater, regardless of wave length.

Believing that the dosages in common use for treatment of plants were much too large, we have used very small doses. The intensity of the radia-

tions used is expressed in Röntgen units measured with a Wulf ionometer,<sup>2</sup> the measurements being taken in air without the effect of back-scattering of the beam by solid material. We are indebted to DR. PAUL C. HODGES, Röntgenologist of the University of Chicago, for the calibration of our instrument, and for many helpful suggestions.

In these preliminary experiments we are using about 100 pk. KV., 5 ma., 1-mm. aluminum screen. Under these conditions the instrument delivers about 38 r-units per minute at a point 30 cm. from the target, the distance used in these experiments. Our experimental material is exposed on cellucotton pads in glass dishes resting on a lead-covered table. It undoubtedly received slightly higher doses than were computed in air because of a slight amount of back-scattering of the radiations. But the computation of the dose in air is a standard method of measuring the dosage. In some instances our best results have been obtained with 1 minute or less, a total of 30-40 r-units. In most cases maximum stimulation has been obtained with not more than 2 or 3 minutes; and with 4 or 5 minutes the effect is already one of retardation of growth.

It is evident at once that investigators who have been using from one to ten erythema doses as light doses, are using extremely heavy doses. The erythema dose is a rather rough unit of measurement, and may be defined as that dose of x-rays that just fails to produce a detectable change in the normal human skin. It is at best a vague designation, but is still much used. It seems much better to adopt the more accurate r-unit. It is generally accepted that the physical equivalent of the erythema dose is approximately 600 r. The Holz knecht is also used in expressing x-ray doses, and this is approximately 120 r.

The optimum dosage for different kinds of plants is probably specific, and must be determined by experiment for each species and varietal strain. A number of common plants seem to respond best to dosages between 30 and 120 r.

### Methods

In order to make it possible to repeat our procedure, the details of preparation of the seeds for treatment are given. Seeds of such plants as corn, wheat, oats, and sunflower have been used. They are placed for 24 hours in a moist chamber upon a layer of cellucotton saturated with distilled water, and kept at a temperature of about 22° C. The seeds are used without sterilization, and lie in contact with the wet substrate on one side, and in contact with moist atmosphere on the other side. They are not

<sup>2</sup> Small-chamber instruments of this sort are intended primarily for use with higher voltages and are somewhat inaccurate at lower voltages. Eventually the calibration will be checked with large-chamber instruments that are relatively insensitive to voltage change.

submerged during the period of preliminary imbibition and germination. At the end of 24 hours the seeds of all four species show incipient germination. The radicles protrude through the pericarps and enable one to know that the seeds are alive. At this stage the material for treatment and for controls is selected. Twenty or more seeds as nearly at the same stage of germination as possible (estimated by equal length of protruding radicles) are chosen and divided into two lots. One lot is left untreated, the other is placed upon fresh saturated cellucotton and treated at once for 1-5 minutes. Optimum effects are often obtained with 1, 2, or 3 minutes of treatment, according to species. Sunflower seems best at 3 minutes, corn possibly at 2 minutes, and some varieties of wheat at 2 minutes. In some cases wheat gives good results at 30 to 45 seconds or 1 minute. As soon as the raying is completed, controls and treated seeds are both planted in the same type of soil, or in sand culture, or on fresh saturated cellucotton in a moist chamber, depending upon the nature of the experiment. In the case of respiration experiments, controls and treated seeds are placed on a wet substrate in the respirometer immediately after treatment. During treatment the glass covers of the moist chambers or petri dishes are removed so that the only screen is the metallic aluminum screen. In the case of sunflower seeds the pericarps of the fruits are removed before treatment. They are also removed from the controls before planting. We have tried to avoid any differences except that of the treatment itself. Selection of seeds is practiced only to obtain material of uniform physiological activity for the controls and treatments.

## Results

### WHEAT

The first tests with Marquis spring wheat indicated that it is sensitive to small doses of x-rays. The treated plants were decidedly more vigorous than the controls when the period of exposure was from 45 seconds to 1 or 2 minutes. By the time the plants were several weeks old (in soil culture), the treated individuals were taller and of ranker growth. The greatest difference was in the degree of tillering. The untreated plants showed 50 per cent. with one tiller each, while the treated plants showed 100 per cent. with two tillers each. Figure 1 shows the general appearance of the plants on September 17, after several weeks of growth.

Tests with Minhardi and Trumbull wheat gave us the impression at the time that the hardier variety (Minhardi) was less easily influenced by x-rays. The Minhardi wheat in the first tests seemed to show little stimulation, while Trumbull, a moderately hardy variety, showed plainly that its early development was hastened by treatment, but not so much as the Marquis spring wheat. At the present time we are not certain as to the order of

these varieties with reference to degree of stimulation.<sup>3</sup> It is possible that varieties more stable toward cold treatments may also be more stable toward x-ray action. We believe the dosage is specific for each variety, and that a longer treatment may possibly be required by the hardier varieties to produce a given amount of stimulation.

### CORN

The most interesting results were obtained with Madison Yellow Dent corn. It was noted that grains which had been treated emerged from the soil more rapidly. On September 22, seeds which had been imbibing water for 24 hours were treated 1-5 minutes, one series screened by aluminum, another treated without metallic screen. A third series, untreated, served as controls. Five days later the seeds treated through the screen showed 84 per cent. of emergence; the unscreened treated seeds showed 72 per cent. ;



FIG. 1. Influence of x-rays on growth of wheat: Pot at left rayed 1 minute; at right, 45 seconds. Controls in middle pot. For other conditions see text.

and of the controls only 60 per cent. had emerged. Treated seeds kept in petri dishes always showed a more rapid elongation of coleoptiles than untreated seeds. We have removed such coleoptiles from the seeds at the end of three days and determined the fresh and dry weight of the coleoptiles. Treated seeds showed from 5 to 26 per cent. greater fresh weight than the controls, and from 3 to 16 per cent. greater dry weight. This suggests the possibility that there is a more rapid utilization of the endosperm reserves in seeds that have been treated.

When the treated corn seeds were grown for a few weeks, some very important differences were noted. Figure 2 shows corn grown from seeds treated 1-5 minutes under an aluminum screen. While the growth differences are visible, and somewhat irregular, the main differences in this set are not visible to the eye in the photograph. The plants treated for

<sup>3</sup> Work on these varieties of wheat is being continued by Miss BESSIE ZABELIN.

TABLE I  
INFLUENCE OF X-RAYS ON GROWTH OF CORN

TREATMENT	STEM DIAM.		FRESH WT. ROOTS		DRY WT. ROOTS		FRESH WT. TOPS		DRY WT. TOPS		CHLOROPHYLL* FRESH WT.			CHLOROPHYLL* DRY WT.		
	mm.	%	gm.	%	gm.	%	gm.	%	gm.	%	%	mg./cm. <sup>2</sup>	mg.	%	%	%
Control	5.63	100.0	54.7	100.0	5.11	100.0	49.7	100.0	4.655	100.0	0.1006	0.0125	5.03	0.874	100.0	
1 min.	5.85	103.9	58.8	107.5	4.76	93.1	65.4	131.6	6.03	129.5	0.1341	0.0163	6.70	1.22	139.6	
2 min.	6.73	119.5	54.7	100.0	4.79	93.7	86.1	173.2	7.67	164.7	0.1688	0.0219	8.44	1.43	163.6	
3 min.	6.15	109.2	54.2	99.0	3.69	72.2	63.9	128.5	5.85	125.6	0.1517	0.0201	7.58	1.34	153.3	
4 min.	6.47	114.9	60.6	110.8	4.80	93.9	86.6	174.2	7.30	156.8	0.1573	0.0205	7.86	1.57	179.6	
5 min.	5.45	95.2	62.2	113.7	5.66	110.7	65.1	130.9	6.44	138.3	0.1407	0.0178	7.03	1.21	138.4	

\* Chlorophyll determinations according to Guthrie, made by Mr. G. B. Ulvin.

short periods (1-3 minutes) had thicker stems than the controls, or those treated 5 minutes. The treated plants looked and felt slightly more succulent, and were darker green in color. The fresh green weight of the tops was obviously greater in the treated plants than in the controls. Without detailed discussion we present in table I such differences as were measured. The chlorophyll differences need further investigation, as this darker green color was not noticed in the oats, wheat, and sunflowers.

The irregular growth of the 3-minute plants in figure 2 may have been caused by a defect in the instrument which was not discovered and corrected until after several lots of seeds had been treated. In table I the most important data are those on dry weight increase (column 11) and those on chlorophyll increase (column 16).



FIG. 2. X-rays and the growth of corn. Control at the left. Time of treatment in minutes indicated on the pots. For other conditions see text.

In table II are presented data on the moisture content of the roots and stems. While the differences are small, they affect roots and tops alike.

TABLE II  
WATER CONTENT OF X-RAYED CORN PLANTS

TREATMENT	ROOTS		TOPS	
	DRY WEIGHT	WATER	DRY WEIGHT	WATER
	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>	<i>per cent.</i>
Control . .	9.34	90.66	8.58	91.42
1 min. . . .	8.09	91.91	8.32	91.57
2 min. . . . .	8.75	91.25	8.03	91.97
3 min. . . . .	6.78	93.22	8.25	91.75
4 min. . . . .	7.92	92.08	7.87	92.13
5 min. . . . .	9.14	90.86	9.06	90.94

With light doses, the dry weight percentage decreases and the water content increases. Even these small differences are large enough so that the practiced eye and touch can detect the greater succulence of the plants from seeds treated for 1–3 minutes.

### OATS

Only one experiment has been performed with oats. The seeds were from a laboratory sample without name. The increased growth of treated seeds was irregular, as in the case of corn, but plainly visible in all of the treated material. Figure 3 shows the results with plants from seeds

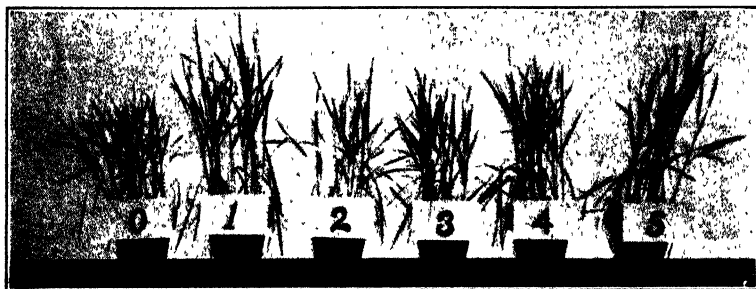


FIG. 3. X-rays and the growth of oats. Control at the left. Time of treatment in minutes indicated on pots. For other conditions see text.

rayed through a 1-mm. aluminum screen at 30 cm. for the periods of time marked on the pots. A defective contact in the machine is believed to have been responsible for the irregular behavior at 2, 3, and 4 minutes, but even these showed increased growth in height and thicker culms than the controls.

### SUNFLOWER

The sunflowers were treated after the x-ray machine had been repaired. In figure 4 the controls and treated plants show an excellent curve of height growth. In the photograph the 2-minute and 4-minute plants were omitted. They were perfectly intermediate between 1 and 3 minutes, and 3 and 5 minutes respectively. The 10-minute plants were rayed without the screen. These unscreened plants show the symptoms of burning described by JOHNSON (2). The leaves are asymmetrical, distorted, pocked as if they had mosaic, and the plants are greatly stunted. The screened plants show none of these ill effects; leaves are normal in every way, and growth more rapid. The group of plants rayed 3 minutes blossomed first, indicating a slight shortening of life history by the treatment.

Some attention has been given to the carbohydrate metabolism and respiration of treated seeds. Under the methods we are using, a slightly more rapid liberation of sugar is detectable from the reserves of corn, and a

slightly more rapid respiration of rayed seedlings. The increases are not very striking, and we feel that the data are too meager to be published at present. It seems hardly possible that the increased rate of emergence of seedlings, increased rate of growth, etc., could take place without some increase in respiration rate. This may be controlled in part by the concentration of sugar in the protoplasmic environment. The first tests on diastatic activity, however, showed distinct depression of the enzyme by x-ray treatment. Much more extensive tests must be made on sugar concentration, respiration, and enzyme activity with material more favorable than corn for this purpose.

### Conclusion

From the results obtained in these preliminary experiments it is concluded that if the x-rays are properly filtered to decrease the intensity of the beam, or to decrease the proportion of the longer radiations, and if the quantity of energy used is adjusted to the specific requirements of the



FIG. 4. X-rays and growth of sunflowers. Control at left. Time of treatment in minutes indicated on pots. Plants at right unscreened. For other conditions see text.

plants by control of the duration of radiation, and of the voltage and amperage used, plants can be stimulated to show increased growth rates.

### Summary

1. A few preliminary experiments are described which indicate that under appropriate conditions of treatment, x-rays produce stimulative effects upon plant growth. Wheat, corn, oats, and sunflower seedlings have been used.

2. The seeds were treated in an early stage of germination after soaking for 24 hours in a closed moist chamber on a substrate of cellucotton saturated with water. The seeds are not submerged during soaking, but are wet on one side, and in contact with air.

3. The conditions which we believe necessary for such stimulative action are: the use of metallic screens, high voltage and low amperage, and brief exposures. The total dosage for stimulation does not much exceed 100 r-units. Even with the 1-mm. aluminum screen sunflowers given 150–200 r-units were overtreated. Optimum growth occurred with about 115 r-units (3 minutes).

4. There is some evidence of increased sugar content and increased respiration of treated seedlings.

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# DECREASING HARDINESS OF WINTER WHEAT IN RELATION TO PHOTOSYNTHESIS, DEFOLIATION, AND WINTER INJURY<sup>1</sup>

S. T. DEXTER<sup>2</sup>

(WITH THREE FIGURES)

The hardening of plants with the approach of the winter season has been the subject of numerous studies; the decreasing hardiness of plants with the approach of spring has received little attention. DEXTER (1, 3) and DEXTER, TOTTINGHAM, and GRABER (4, 5) have made use of methods of studying hardiness which involved the principle of changed permeability and electrical conductivity of tissues injured by freezing. Plants were hardened under various controlled conditions and it was found that they hardened poorly or not at all in the dark, when low in organic reserves; plants in the same condition hardened well if given opportunity for photosynthesis at low temperatures. If the conditions during hardening were such that material vegetative extension occurred, hardening was in general lessened thereby. If, during part of the day and especially at night, the respiration rate of the plants was raised by elevating the temperature, hardening was inhibited (see also PELTIER and TYSDAL 6). In general the development of hardiness was favored by environmental conditions which would seem to tend toward the accumulation or conservation of the organic food supply.

This paper deals with the application of these general principles to the problem of the return of the plant to the less cold-resistant condition. There appears to be little if any description of work done in regard to the rapidity of development of this condition, or the factors influencing it.

## Experiment 1

Samples of two varieties of winter wheat were brought into the greenhouse at monthly intervals throughout the winter. The varieties, Minhardi (very hardy) and Wisconsin Ped. no. 2 (a hardy Turkey selection), were seeded in 12-inch pots and placed in soil out-of-doors, on September 23, 1931. The soil was of good fertility. The rather loose seed bed appeared to favor a rapid and somewhat more vegetative growth than was found in plants seeded in adjacent unworked ground. The plants in the pots were somewhat less hardy than those in the firm soil, but they appeared to be in no way unusual for winter wheat.

The weather during the winter was generally mild, although the temperature frequently went below freezing. Figure 1 shows the temperature

<sup>1</sup> Contribution from the Hull Botanical Laboratory, University of Chicago.

<sup>2</sup> National Research Council Fellow in the Biological Sciences.

record for the period. Up to December 18, when the first samples were taken, there had been no weather cold enough to kill the leaves of the wheat, and both varieties were in excellent condition, with a large photosynthetic area. During the interval from December 18 to January 18, no weather conditions occurred which would kill the wheat leaves, and on the latter date the foliage was still as green as on December 18. During January 29 to 31, however, the temperature dropped sharply, with no snow cover, and the plants were almost completely defoliated by the cold. A period of warm weather followed and new leaves promptly developed. These leaves, about 1 inch in length, were on the plants at the time of the February 18 determinations. In the greenhouse these plants (February 18 samples) continued to grow with rapid foliar elongation, whereas on the previous dates little or no vegetative extension could be seen. In early March the temperature again became sufficiently low to kill the leaves by freezing, although there was some snow cover accompanying the low temperature. This cold

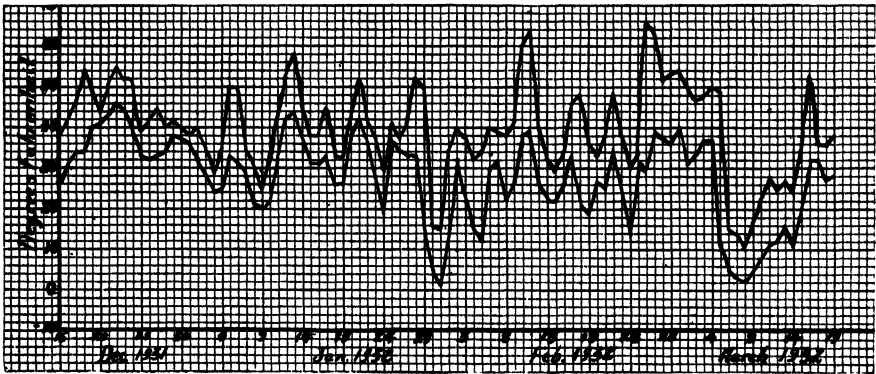


FIG. 1. Daily maximum and minimum temperatures, in ° F., at Chicago, Illinois, for the dates indicated.

period was of considerable duration and the ground became deeply frozen for the only time during the winter. The new growth (since February 1) was completely killed and the Wisconsin Ped. no. 2 plants were obviously severely injured by the cold. In the greenhouse, following March 17, renewal of leaves on Minhardi plants was rapid; only a few of the Wisconsin Ped. no. 2 plants recovered.

To study the condition of these plants throughout the winter, the following scheme was used. On December 18, January 18, February 18, and March 17, one pot of each variety was brought from the field into the greenhouse, where they were exposed to the daylight prevailing on the respective dates, at a temperature held at about 62° F. The hardiness of the plants was determined when they were brought in, again after two days in the

greenhouse, and then after seven days in the greenhouse, by the freezing-exosmosis test used in previous experiments (2).

Some difficulty was anticipated in obtaining relatively constant conditions during the period allowed for decreasing hardiness. The weather, however, happened to be favorable in that regard. There were no ice crystals in the pots on December 18 when they were brought in, although the soil had been very recently frozen. The same was true on January 18. On the night of February 17 the temperature dropped, freezing the soil to a depth of 1 to 2 inches. During the cold weather early in March, the soil froze to a depth of about 12 inches, but a day or two of warm weather preceding March 17 had thawed the upper 3 or 4 inches. Little difficulty was experienced in keeping the greenhouse at the approximate temperature of 62° F.

The samples were prepared as usual: duplicate 2.5-gm. samples of crowns were frozen in tubes at -15° C. for 2 hours in an alcohol-slush bath, thawed for 30 minutes at 2° C., and 25 cc. of water at 2° C. were then added to each tube. Exosmosis continued for 16 hours at 2° C., when the conductivity readings were made. (In some cases, 1-gm. samples were used with 10 cc. of water, with other procedures the same. This did not appear to alter the values.)

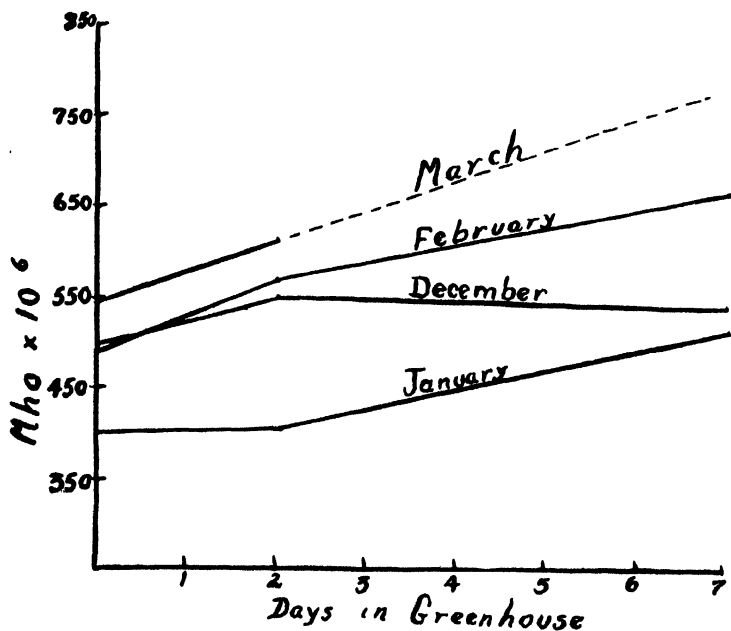
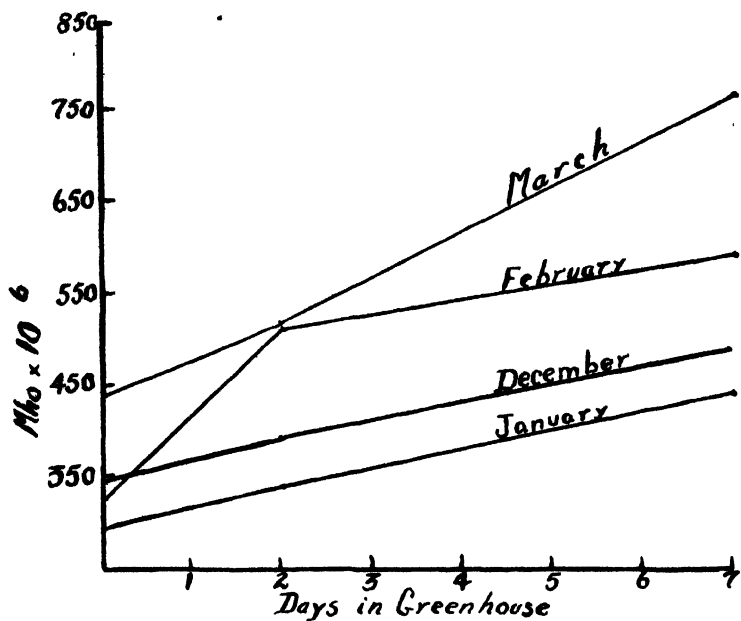
Table I shows the results secured on the different dates. Figures 2 and 3 show the data in graphic form for the two varieties.

TABLE I

SPECIFIC CONDUCTIVITIES ( $\times 10^6$ , 2° C.) EXPRESSED IN RECIPROCAL OHMS OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR 2 HOURS AT -15° C. INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

VARIETY	DATE BROUGHT IN	DAYS IN GREENHOUSE AT 62° F.		
		0	2	7
Minhardi Wis. Ped. no. 2	December 18	{ 342 496	391 548	490 539
	January 18	{ 294 392	342 400	443 515
	February 18	{ 329 493	511 562	592 658
	March 17	{ 439 548	516 605	767 Dead, no sample

On every date and at every stage of decreasing hardiness, Minhardi was found to be hardier than Wisconsin Ped. no. 2. On December 18 the varieties were somewhat hardy, and their hardiness decreased rather slowly in the greenhouse, being far from complete at the end of one week at 62° F.



FIGS. 2, 3. Graphs showing specific conductivities ( $\times 10^6$ ) in reciprocal ohms of extracts from two varieties of winter wheat. Fig. 2 (above), Minhardi; fig. 3 (below), Wisconsin Ped. no. 2.

On January 18 both varieties were distinctly hardier than in December, and decreased in hardiness still more slowly. These plants had had abundant opportunity for photosynthesis during the intervening month, had suffered no defoliation nor winter injury, and had made no visible vegetative extension. It seems highly probable that photosynthesis had more than balanced respiration, since there was little or no snow, the plants only occasionally frozen, and soil water available.

The sharp frost at the end of January introduced another feature. Winter injury at this time was considerable, although there was no evidence that any plants of either variety were killed outright. The warm weather following stimulated prompt elongation of the inner leaves, even though the soil was scarcely thawed. This growth no doubt used a part of the organic reserves of the plants and some decrease in hardiness was found on February 18, as the conductivity values show. In the greenhouse following this date, vegetative growth continued rapidly, together with a pronounced decrease in hardiness. In the same way the cold weather during the first part of March killed the extensive new growth made since the end of January. Not only that, but it killed as well the leaf sheaths and meristematic regions of many of the older leaves. As a result of this injury the crowns, which in December were rather large in diameter, became smaller following each freezing. Finally but one or two leaves were left which were capable of elongation. The contrast was clearly seen in the hardier Minhardi, grown in the adjacent firm ground. Here the tendency toward radial depletion of the crown tissue was very markedly less, and in the spring more leaves could grow. The depletion of the storage in these two ways (by growth of new leaves and by killing of partially depleted tissues) resulted in weakened and non-hardy plants which rapidly decreased in hardiness in the greenhouse. Thus, on March 17 the remaining leaves of many of the Wisconsin Ped. no. 2 plants had been killed. In some cases a slight growth of perhaps half an inch was made, but most of these leaves finally failed to survive. In no case was growth from small axillary buds observed; that is, killing of the somewhat expanded leaf tissue at the crown appeared to kill the plant. Following March 17, decrease in hardiness was rapid, and almost complete in one week. Most of the Wisconsin Ped. no. 2 plants died, and the Minhardi plants showed rapid foliar elongation in the greenhouse.

It is interesting to note that on February 18 the plants of both varieties, as brought in from the field in an almost completely defoliated condition, were hardier than they were on December 18. They decreased in hardiness, however, much more rapidly in the week following February 18 than following December 18 (or January 18). This appeared to be associated with the two factors previously discussed. Growth of the leaves was not evident following December 18 or January 18, whereas it was rapid following

February 18. The plants brought in on February 18 had been almost completely defoliated on January 31, and had made about 1 inch of growth since that time; in the greenhouse these new leaves elongated quickly. Furthermore, the crowns had then been sufficiently injured so that some loss of leaf sheath tissue through killing had occurred.

### Experiment 2

On January 22, lumps of soil containing plants of Minhardi (hardy) and Trumbull (rather tender) wheat were brought into the cold room from the field plats and stored at a temperature of 31°–36° F. The lumps of soil were sometimes partly frozen during the hours in the dark and sometimes completely thawed during illumination. One set of plants received light for 8 hours each day from a 100-watt lamp suspended about 15 inches above the plants. Another set was kept in darkness. After about a month the two sets were tested for hardiness, to see whether the plants would lose in hardiness more rapidly in darkness than in light. Table II shows that this is the case with both varieties. The samples stored in darkness at a temperature slightly above freezing showed marked elongation of etiolated leaves. This growth, combined with the lack of opportunity for photosynthesis, seems responsible for the rather definite decrease in hardiness observed. Trumbull plants decreased in hardiness more than Minhardi ones under this treatment. This might well be expected from the work of NEWTON and ANDERSON (7), who showed that the respiration rates of tender varieties of wheat are higher at low temperatures than are those of hardy varieties.

Part of the plants in both the light and the dark were defoliated on February 18 and kept in the cold room as before. In each case new leaves developed, although in some cases the lumps of soil were almost solidly frozen during the entire experiment. When these plants were tested for hardiness about a month later, plants which had been defoliated were more tender than undefoliated ones in each case.

On February 18, Minhardi plants in the field plats were defoliated by clipping the leaf blades with scissors. These plants promptly began foliar development. On March 17, following a period of cold weather, it was evident from the appearance of the plants that they had been severely injured by the cold. Every trace of the new green leaves was gone, and the plants were withered back to short stumps at the crown. The adjacent plants that had not been defoliated still had an abundance of green leaves. Samples were taken for the freezing-exosmosis test, and as the values in table II show, the defoliated plants were found to be much more tender than the corresponding undefoliated plants. The later development of these two sets showed that, while most individuals of both sets survived,

the defoliated plants were weak and slow to make new growth. While not winter-killed, they were certainly winter-injured.

TABLE II

SPECIFIC CONDUCTIVITIES ( $\times 10^6$ , 2° C.) EXPRESSED IN RECIPROCAL O $\overline{\text{HMS}}$  OF EXTRACTS OF CROWNS OF WINTER WHEAT FROZEN FOR 2 HOURS AT -15° C. INTERVAL OF 16 HOURS ALLOWED FOR EXOSMOSIS AT 2° C.

VARIETY	TREATMENT	READINGS TAKEN FEBRUARY 18
Minhardi {	Outside, no serious injury by cold . . . . .	266
	Inside, light, cold for one month . . . . .	241
	Inside, dark, cold for one month . . . . .	292
Trumbull {	Outside, injured by cold on January 30 . . . . .	490
	Inside, light, cold for one month . . . . .	430
	Inside, dark, cold for one month . . . . .	567
		READINGS TAKEN MARCH 17
Minhardi {	Outside, not defoliated with scissors . . . . .	343
	Outside, defoliated with scissors on February 18 . . . . .	502

### Discussion

The decrease in hardiness of winter wheat plants, so that they are more readily injured by cold weather, is a matter of considerable agronomic importance. The retention of hardiness seems, from these experiments, to be dependent upon the preservation of an adequate supply and concentration of organic foods. This supply is ordinarily depleted by respiration. If there is opportunity for the supply to be renewed through photosynthesis, as was the case in these experiments from December 18 to January 29, the plants may not become progressively more tender. Defoliation of the plants, whether artificially or by freezing injury, does not seem to be detrimental to their hardiness, as the conductivity values for February 18 show. Defoliation by either means, however, appears markedly to stimulate production or elongation of new leaves, which is provocative of rapid decrease in hardiness, presumably because of the labilization and use of the organic food reserves. The temperature at which this elongation takes place may be a decided factor in the effect produced. Thus the leaves of plants kept in darkness at about 0° C. elongated considerably in the course of a month, but without especially rapid decrease in hardiness of the plants. Plants elongating no more (and in fact in some cases imperceptibly, as from December 18 to 25) decreased materially in hardiness when kept at higher temperatures (about 17° C.) for one week. In general it would appear that the maintenance of the hardened condition in winter wheat plants is dependent upon environmental conditions which favor the con-

servation of organic food reserves; that is, which depress respiration and top growth and favor dormancy with continued periods of photosynthesis.

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## FURTHER EVIDENCE THAT BORON IS ESSENTIAL FOR THE GROWTH OF LETTUCE<sup>1</sup>

J. S. MCHARGUE AND R. K. CALFEE

(WITH EIGHT FIGURES)

In a previous report (2) it was shown that boron is essential for the growth of several varieties of lettuce; and that when it was excluded from the mineral nutrient solution, a severe deficiency disease resulted which was characterized by malformation of the more rapidly growing leaves, spotting and burning of the leaf tips, and death of the growing point of the plant. A similar condition of lettuce was described by STONE and SMITH (3) as "top-burn." They considered the cause of the disease to be physiological and promoted by unfavorable surroundings. LE CLERG (1) measured leaf temperatures, but could not establish a relation between temperature and tip burning. The writers have further investigated the disorder, from the standpoint of a nutritional deficiency.

The influence of various boron compounds in preventing the burning of lettuce leaves was studied in both sand and water cultures. Control of the exact boron content of cultures was obtained by the addition of definite quantities of pure boron compounds to boron-free media. A dilute Pfeffer's solution, to which small quantities of manganese, copper, and zinc were added, supplied the basal mineral nutrients for both sand and water cultures. The salts composing the basal solution were proved to be free of boron by spectroscopical examination. Sand was purified by digestion with hot hydrochloric acid, followed by removal of chlorides with distilled water. Distilled water required for the preparation of cultures, growth of plants, purification of sand, and rinsing of containers was condensed in quartz. Porcelain dye pots and special acid-resistant jars with perforated lids were used as containers for the sand and water cultures respectively. No evidence of boron contamination from the use of these containers was observed.

Lettuce seeds were germinated in purified sand, and transferred to sand or water cultures containing, with the exception of boron, all elements known to be essential for plant growth. Severe boron deficiency, as indicated by severe injury to the leaf tips, developed in from two to four weeks, depending upon the variety, amount of light, and type of culture used.

<sup>1</sup> Contribution from the Department of Chemistry of the Kentucky Agricultural Experiment Station.

The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

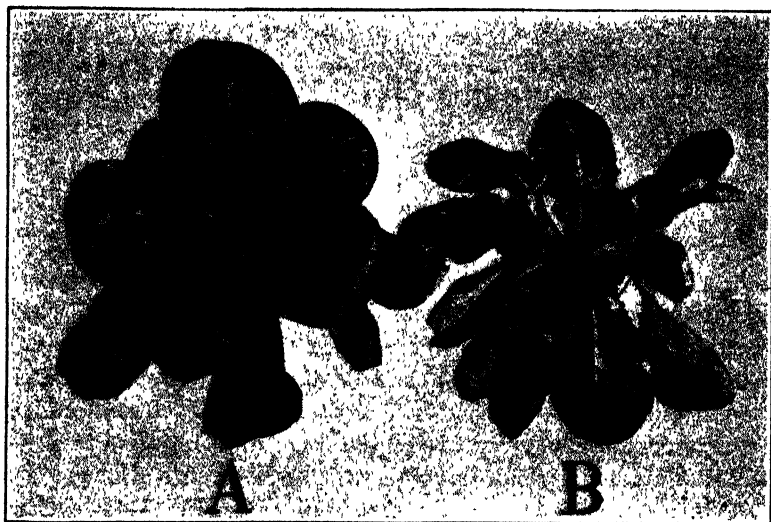


FIG. 1. Effect of boron on growth and development of lettuce plants: *A*, plant grown in sand containing 0.5 p.p.m. boron; *B*, plant not supplied with boron.

The first symptoms of boron deficiency were a retardation of growth and malformation of the younger leaves. Dark spots then appeared on the margin of the growing leaves, usually at the tip. Marginal growth was



FIG. 2. Effect of boron deficiency on leaf development: *A*, leaf from normal plant; *B*, leaf from plant showing severe boron deficiency.

suspended, resulting in a folding back of the leaf tip. Normal and deficient plants at this stage are shown in figure 1. The spots increased in size and number, involving the entire leaf tip and giving it a scorched appearance. Figure 2 shows leaves from normal and deficient plants of the same age. The older leaves are not noticeably affected by the absence of boron; but all young leaves, from those first affected to the growing point itself, are involved, resulting finally in the destruction of the meristem tissue and consequently in the death of the plant (fig. 3).



FIG. 3. Growing point of lettuce plant showing severe boron deficiency.

The addition of a small quantity of boron to the culture before the death of the growing point relieved the condition and resulted in the production of normal leaves from the growing point. The addition of boron compounds after the death of the growing point resulted in growth from lateral buds in the leaf axils. Figures 4-6 show the results of continued additions of boron to the media after the development of severe deficiency, allowing the plant to make very satisfactory growth to maturity. Plants that were retarded in early growth by the absence of boron did not attain the same size or weight at maturity as did normal plants (positive controls).

Small quantities of boric acid, boro-silicate (powdered Pyrex glass), and borates of potassium, sodium, calcium, manganese, copper, and zinc were found to be effective in preventing injury to lettuce leaves.

To ascertain the optimum concentration of boron for the growth of lettuce, cultures in triplicate were treated with boric acid varying the boron



FIG. 4. Lettuce plants in which boron deficiency had caused death of the growing point. Boric acid was added to the one on the left at this time.

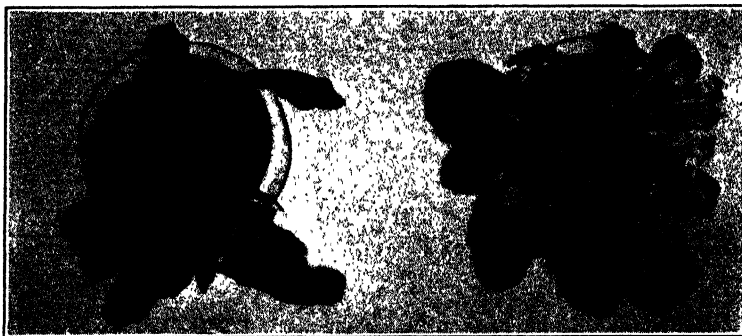


FIG. 5. Same plants as in figure 4, 14 days later. Growth has been resumed from axillary buds in the plant receiving boric acid.



FIG. 6. Same plants as in figure 4, 6 weeks after addition of boric acid to the one on the left.

content by 0.1 p.p.m. from no boron to 1.5 p.p.m. The range between 1.5 and 3.0 p.p.m. was covered by increase of 0.3 p.p.m. boron.

Plants that did not receive boron showed injury shortly after being transferred to the experimental cultures. With 0.1 and 0.2 p.p.m. boron in the medium, plants made much slower growth than those receiving larger quantities, within non-toxic range. The presence of 0.3 p.p.m. boron in the nutrient solution enabled plants to make continued growth until shortly before reaching maturity; then during periods of rapid growth injury would appear on the more rapidly growing leaves. During periods of lower activity, imposed by shading or unfavorable weather conditions, growth would be resumed, with the production of apparently normal leaves. The plants of this group were much smaller than normal plants and did not produce flowers. All plants in the group grown in a boron concentration of 0.4 p.p.m. were free from leaf injury. Florescence occurred although

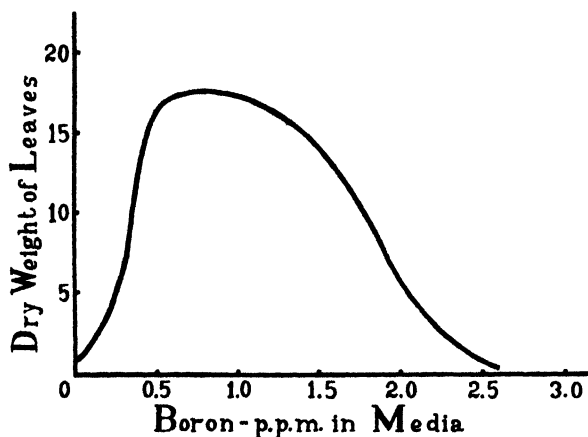


FIG. 7. Dry weight, in grams, of leaves of lettuce plants grown in the presence of different quantities of boric acid.

but few seeds were produced. Quantities of boron between 0.4 and 0.9 p.p.m. in the culture resulted in the production of vigorous, rapidly growing plants with no evidence of toxicity or boron deficiency. Seed pods containing normal seeds were especially numerous on plants grown in concentrations of 0.6 and 0.7 p.p.m. boron. Nine-tenths of a part of boron per million was slightly toxic to lettuce, resulting in perceptible chlorosis of the lower leaves in most plants. The chlorosis was more pronounced with 1.0 p.p.m. boron in the nutrient solution. Boron concentration of 1.2 p.p.m. was decidedly toxic, causing chlorosis and death of the older leaves and large, white, necrotic spots on the edges of mature living leaves. All concentrations of boron from 1.2 to 2.5 p.p.m. produced increasing toxicity, as shown by necrosis and a significant decrease in the size of the plants.

Quantities of boron exceeding 2.5 p.p.m. were fatal to the seedlings. The effects of different quantities of boron on the growth of lettuce as determined by dry weight of the leaves are shown in figure 7. On the basis of dry weight, the optimum concentration of boron in water cultures for the growth of lettuce is 0.7 p.p.m. added in the form of boric acid.

The utilization of various boron compounds by lettuce was investigated in sand cultures to determine the relative amounts that must be present for normal growth. The powdered borates were mixed with purified sand in porcelain dishes before placing in the containers. Other mineral nutrients were added in solution as required through the period of growth. Treatments and results are given in table I.

Additions of boric acid, or the relatively soluble borates of potassium, sodium, and calcium to sand in sufficiently small quantities to avoid toxic effects were insufficient for the later growth requirements of the plants, unless a large volume of medium was used. Normal plants could not be grown in sand cultures of 750 or 1500 gm. without further additions of soluble boron compounds during growth. Cultures of 5 kg. containing sufficient soluble boron compounds to produce normal plants were not toxic to plants started during the early summer, but were decidedly toxic to seedlings started during periods of more limited light intensity and shorter daily exposure. The quantity of soluble borate in cultures of 10 kg. was decreased to a content that was non-toxic to seedlings at any period of the year without the appearance of deficiency symptoms in later growth.

The inclusion of 0.0025 gm. of boron as manganese borate in sand cultures was sufficient for the growth to maturity of the lettuce plant. Relatively large quantities of manganese borate were not toxic to plants in cultures having reaction exceeding pH 6.6. Increase in the acidity of cultures resulted in high concentrations of manganese borate becoming toxic. Toxicity resulting from an excess of manganese borate was due to the boron ion. The addition to sand cultures of sufficient copper borate to supply the plants' boron requirements throughout the period of growth resulted in toxic concentrations of the copper ion.

Boro-silicate in the form of powdered Pyrex glass was found to be the most satisfactory source of boron for sand cultures. Quantities sufficient for the successive growth to maturity of several plants were included in the medium without toxicity or the development of deficiency symptoms. Slight changes in the reaction of the culture did not affect the solubility of the boro-silicate.

The ratio between the quantity of boron available and the quantity absorbed and its relation to the physical condition of the plants was ascertained by determining the boron content of plants grown in water cultures

TABLE I

EFFECTS OF DIFFERENT QUANTITIES OF BORON COMPOUNDS ON GROWTH OF LETTUCE

COMPOUND	BORON PER KG. SAND	EFFECT OF BORON COMPOUND ON PLANT
	<i>gm.</i>	
Boric acid	0.0005	Deficiency severe
	0.0010	Deficiency severe
	0.0015	Slightly toxic, final deficiency
	0.0020	Toxic
Potassium borate	0.0005	Deficiency severe
	0.0010	Deficiency severe
	0.0015	Deficiency; some toxicity
	0.0020	Toxic
Sodium borate	0.0005	Deficiency severe
	0.0010	Deficiency severe
	0.0015	Deficiency
	0.0020	Toxic
Calcium borate	0.0010	Deficiency severe
	0.0015	Deficiency
	0.0020	Slightly toxic at first; good growth
	0.0025	Toxic
Manganese borate	0.0025	Good growth
	0.0075	Very good growth; plant normal
	0.0100	Slightly toxic
	0.0150	Boron and manganese toxic
Copper borate	0.0010	Deficiency severe
	0.0025	Cu. slightly toxic
	0.0050	Cu. toxic; severe
	0.0075	Seedlings killed
Zinc borate	0.0010	Deficiency severe
	0.0050	No deficiency or toxicity
	0.0100	Zn. toxic, slight
	0.0150	Zn. toxic, severe
Boro-silicate (powdered Pyrex glass, 40 mesh)	10.0	Plant normal
	25.0	Plant normal
	50.00	Very slightly toxic
	100.00	Definitely toxic

at constant concentrations of boron. The boron content of normal lettuce varied between 25 and 50 p.p.m. boron of the moisture-free tissues. All plants containing less than 20 p.p.m. boron showed some degree of defi-

ciency. Boron toxicity occurred in all plants containing more than 60 p.p.m. boron.

Figure 8 shows the effects of different concentrations of boric acid in

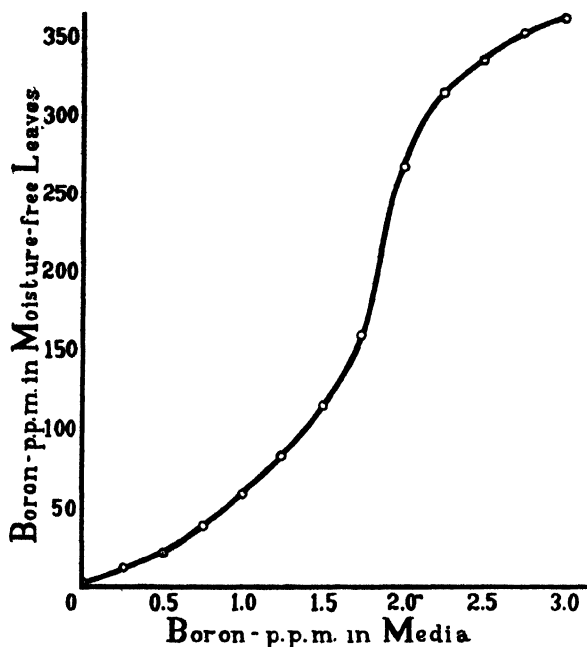


FIG. 8. Absorption of boron from nutrient solutions of different concentrations of boric acid.

the nutrient solution on the absorption of boron by lettuce. The concentration in the leaves depended upon the concentration of soluble boron in the solution. Slight increases in the boron content of a solution exceeding toxic concentrations resulted in large increases in the boron content of the leaves. Additions of boric acid greater than the quantity sufficient to kill the seedlings rapidly caused a slightly greater increase in the boron content of the leaves than did the minimum lethal concentration, indicating a relation between the concentration of boron in the medium and the rate of absorption by the plant.

### Summary

1. A deficiency disease of lettuce resulting from an insufficiency of boron is described and illustrated.
2. The effectiveness of several boron compounds in preventing or correcting the deficiency disease is given.
3. The concentrations of soluble boron compounds that result in boron deficiency, normal growth, and toxicity were ascertained.

4. The physical condition of the plant was modified by the concentration of soluble boron in the nutritive solution.

5. The effect of the less soluble boron compounds was modified by the reaction of the culture, the volume, and, through the plant itself, by climatic and seasonal conditions affecting the rate of growth.

6. Boro-silicate was found to be the most satisfactory compound for incorporation in sand cultures.

7. Increase in the boron content of the nutrient solutions up to concentrations that rapidly resulted in the death of the seedlings produced increasingly greater concentrations of boron in the leaf tissue.

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# RAPID COLORIMETRIC DETERMINATION OF POTASSIUM IN PLANT TISSUES<sup>1</sup>

V. H. MORRIS AND R. W. GERDEL

## Introduction

One of the most important factors limiting studies of the mineral nutrition of plants is the time and labor consumed in the analytical processes. Nowhere in the field of plant physiology is the need for rapid, convenient, and accurate methods more keenly felt. With such material as corn, the great variability in the composition of individual plants of even nominally pure lines is such as to necessitate considerable replication of samples in order to obtain analyses which are representative of the population.

Of the plant constituents usually considered in mineral studies, potassium is probably the most difficult and tedious to determine by the conventional methods. Both the chloroplatinate and perchlorate methods are time-consuming and involved. In recent years, however, a method based on the precipitation of the potassium by sodium cobaltinitrite has met with increasing favor, particularly in blood and urine analyses. A study of the adaptability of this method to samples of plant tissues, of which corn may be considered representative, is reported in this paper.

## Method

The principle of the method is based upon the precipitation of potassium as the double salt, potassium sodium cobaltinitrite, of the general composition  $K_2NaCo(NO_2)_6 \cdot H_2O$ . The quantity of the precipitate may be determined by any of the three common analytical procedures, gravimetric, volumetric, or colorimetric. Since colorimetric methods are notably time- and labor-saving, while retaining a reasonable degree of accuracy, such a procedure seemed most desirable.

As with most methods for determining potassium, the presence of ammonia in the sample interferes by forming a relatively insoluble precipitate with the reagent. Ammonia must accordingly be removed from samples containing enough to introduce serious error. Seasonal studies have shown that the quantity of ammonia present in corn tissue at any time is not sufficient to introduce appreciable error. This is probably true also of most plants, except perhaps when they are grown under unusual environmental conditions.

<sup>1</sup> Investigations cooperative between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture and the Department of Agronomy, Ohio Agricultural Experiment Station.

For use with corn the method must be adaptable to two kinds of samples, sap expressed from fresh, succulent tissues, and solutions of the ash from dry, mature tissues. As determined by the chloroplatinate method, sap expressed from different tissues of the corn plant contained from 1 to 6 mg. K per ml. Ashed solutions contained comparable quantities in 25 ml. of the solution as prepared for analysis.

Three procedures for colorimetrically measuring the quantity of the precipitated potassium were investigated: (a) The method suggested by EMMERT (1), based upon measuring the change in strength of the precipitating reagent. With us the method appeared incapable of giving the desired degree of accuracy. (b) Methods based upon determining the quantity of nitrite present in the precipitate. Several of the methods described by YOE (4) were tried. The principal objection to these methods is that they are very sensitive to small quantities of nitrites, thus making it necessary to use very dilute solutions in which to develop the color. These methods are open also to the theoretical objection that the nitrite radical may be considered relatively unstable. (c) Methods based upon estimating the quantity of cobalt in the precipitate. The method described by YOE (4), involving the use of  $\alpha$ -nitroso  $\beta$ -naphthol, was open to the same objection as that to the nitrite methods,—the necessity of developing the color in too dilute solutions. The method suggested by JACOBS and HOFFMAN (2) was then tried and found to be well adapted to the material in hand. This method is based upon the development of a brilliant emerald green color by the addition of ferrocyanide to a solution containing choline and a cobaltous salt.

### Procedure

The precipitation and determination of potassium were carried out as follows:

The expressed sap, obtained as described in a previous paper (3), was cleared by warming to 55° in a water bath, shaking with about 1 gm. of carbon black per 100 ml., and filtering. To 1 ml. of the clear filtrate in a 50-ml. lipped centrifuge tube were added 2 ml. of EMMERT's (1) sodium cobaltinitrite reagent.<sup>2</sup> The tube was allowed to stand for 15 to 20 minutes, with occasional shaking. About 10 cc. of 70 per cent. alcohol were added from a wash bottle and the tube centrifuged for several minutes at moderate speed. The supernatant liquid was poured off, allowing time for

<sup>2</sup> EMMERT's sodium cobaltinitrite reagent: Dissolve 5.1666 gm. of pure sodium cobaltinitrite in 40 ml. of distilled water; dissolve 27 gm. of potassium-free sodium nitrite in another 40 ml. of distilled water; pour the solutions together, add 6 ml. of glacial acetic acid, and make up to 100 ml. with distilled water. One ml. will precipitate about 10 mg. of K as  $K_2NaCo(NO_2)_6 \cdot H_2O$ . The solution evolves gas when first prepared but this does not impair its value.

thorough drainage. This washing process was repeated twice more, using about 10 cc. of 70 per cent. alcohol each time. After the final centrifuging, the precipitate was washed into a 100-ml. volumetric flask with water and dissolved by heating on a steam plate. After cooling to room temperature the solution was made up to volume and shaken. Twenty-five ml. of the solution were pipetted into an Erlenmeyer flask, 5 cc. of a 1 per cent. choline hydrochloride solution and 5 cc. of a 2 per cent. potassium ferrocyanide solution being added with shaking. A brilliant emerald green color develops instantly, the intensity of which is directly proportional to the quantity of cobalt present. The color was compared in a Klett colorimeter with a standard prepared by precipitating a solution of KCl containing 2 mg. K per ml. and carrying it through the same procedure as with the unknown. This method of standardization is necessary, since it has been shown by previous workers that the composition of the precipitate with respect to the sodium content is influenced somewhat by the relative quantities of potassium and precipitating reagents.

With the dried tissues, 2.5-gm. samples were ashed with sulphuric acid, dissolved in dilute HCl, filtered, and diluted to 250 ml. Twenty-five ml. of this solution were evaporated to dryness in a 100-ml. beaker, 1 ml. of water added, and, after the salts were again in solution, 2 ml. of the reagent added. After transferring the precipitate to a 50-ml. centrifuge tube with 70 per cent. alcohol, the procedure was the same as with the sap samples.

### Results

The method has been tried on several materials with results as shown in table I. The recovery of potassium from solutions containing different quantities of potassium acid phthalate and potassium chloride was satisfactory, the error in no case exceeding 0.5 mg. The results of a comparison of the colorimetric method with the standard chloroplatinate method as applied to a series of expressed sap samples are also recorded in the table. The samples were obtained during a study of the comparative potassium contents of a group of corn hybrids, each sample representing the stem tissue of a different hybrid. The samples were taken in duplicate and the values given in the table are the average of single determinations on the duplicates by the method stated. The means for the two methods are in very good agreement. Although the difference between duplicates was rather large in some cases, there is no reason to assume differential variation between samples determined colorimetrically and those determined gravimetrically. Consequently, the difference in the variances for the two methods may be interpreted as indicating that the colorimetric method was at least as precise in these experiments as the chloroplatinate method. Likewise, when the two methods were applied to expressed sap samples from



different tissues of the corn plant taken on the same date, satisfactory agreement between the two methods was obtained.

A comparison of the two methods as applied to ashed samples indicates that the colorimetric method also may be used with this type of sample.

The colorimetric method is not only much cheaper from the standpoint of cost of chemicals and equipment, but is also advantageous from the point of time consumed. At least 24 determinations may be completed in 8 hours, starting either with expressed sap or with ashed solutions, whereas the chloroplatinate method would require 16 to 32 hours for the same number. When working with variable plant material, therefore, the opportunity is afforded for using a greater number of replicates to obtain much greater final precision.

### Summary

The colorimetric procedure of JACOBS and HOFFMAN for determining potassium precipitated as the cobaltinitrite affords a rapid and convenient method for samples of plant tissue. The method compares very favorably with the chloroplatinate method with respect to precision; and the saving of time and labor, permitting the handling of a larger number of replicates, results in a much greater final precision.

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# A NEW METHOD AND INSTRUMENT FOR THE QUANTITATIVE DETERMINATION OF CHLOROPHYLL<sup>1</sup>

R. E. OLTMAN

(WITH THREE FIGURES)

In research problems in plant physiology, it occasionally becomes desirable to make a considerable number of quantitative determinations of chlorophyll. The method by which this is done should be one that is speedy, open to a minimum of error, and not so unwieldy as to be difficult for the general botanist to employ. Many methods for determining the amount of chlorophyll present in leaves or in solutions have been suggested, but only two have been found to be of sufficient accuracy to permit a quantitative comparison with the results of other workers. SCHERTZ (2) has described the colorimetric and the spectrophotometric methods, and has shown the percentage of error prevalent in each.

The colorimetric method depends on the ability of the eye to match depths of color, an operation in which the human eye is notoriously inefficient. In addition, this method does not adequately determine slight differences in color, the limit of sensitivity and accuracy being two decimal places.

The spectrophotometric method depends on measuring the width of the absorption bands of chlorophyll in the red end of the spectrum. This method is neither speedy nor practical for making a considerable number of determinations, since for each determination a separate spectrophotograph must be taken, which requires considerable time. In addition, an expensive spectrograph is necessary, and the aid of an expert physicist is advisable for correctly interpreting the photographs. Like the colorimetric method, it is possible to ascertain concentrations of chlorophyll only to two decimal places.

The purpose in developing the instrument here described was to eliminate the possibilities of personal error in observation, to detect more minute differences than is possible by the preceding methods, and to make the quantitative determination of chlorophyll an easier and simpler process. This instrument will detect differences of 5 mg. between samples, and has a maximum error of 9 per cent. at the lower concentrations and a minimum error of 2 per cent. at the higher concentrations, which errors in most cases affect only the third decimal place.

Figures 1 and 2 illustrate the apparatus. A photoelectric cell (Weston photronic), which requires no external resistances, no amplification of the

<sup>1</sup> Presented at the general scientific sessions of the Ohio Academy of Science, Delaware meeting. April, 1932.

Papers from the Department of Botany, Oberlin College, no. 4.

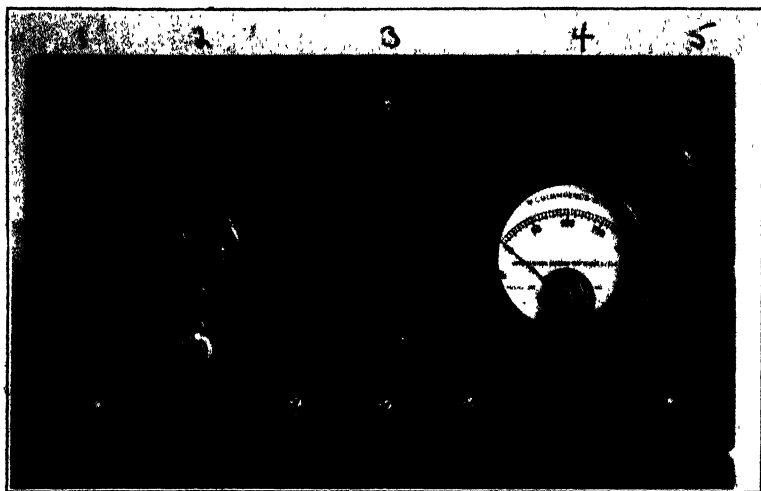


FIG. 1. Exterior view of instrument: 1, binding posts for attachment of 6-volt storage battery; 2, rheostat and switch; 3, sliding panel; 4, microammeter; 5, output binding posts from microammeter.



FIG. 2. Interior view of instrument: 1, binding posts for 6-volt battery connection; 2, 4-ohm rheostat; 3, 2-candle-power automobile bulb; 4, light filter (Corning Lantern shade yellow, no. 349); 5, glass absorption bottle fitted in groove in panel; 6, sliding panel (note guides on sides); 7, photoelectric cell (Weston photronic, Model 594) mounted in UX type radio socket; 8, microammeter (Weston, Model 301); 9, output binding posts from microammeter, so that meter can be used for other purposes without dismantling.

current emitted, and which emits a current in direct proportion to the light energy absorbed on its face (over rather wide limits) is employed. This cell is mounted upright in a UX type radio socket, 15 cm. from a 2-candle-power, 6-volt automobile bulb fitted with a small reflector. A rheostat to vary the light intensity is connected in series with a well-charged 6-volt storage battery and the small automobile lamp. The position of the lamp is so adjusted as to place its center directly opposite the center of the face of the photoelectric cell. The current emitted by the photoelectric cell when exposed to light is measured by a microammeter with a range of 0-200 microamperes, which is connected to the leads of the UX type socket into which the cell is fitted. Between the cell and the lamp a sliding panel of thick wood is interposed, with a 1.5-inch hole bored through it on a direct line with the center of the lamp and the photoelectric cell, so that the light from the lamp is allowed to pass through and strike upon the face of the cell.

On one side of this sliding panel a 2-inch square light filter, transmitting 5600-7000 Å. (Corning Lantern shade yellow, no. 349), is mounted over the hole in the panel. By this means the light from the lamp is all converted to those wave lengths which WURMSER (5) and others have shown to be most strongly absorbed by chlorophyll. The red light is then passed through a small glass absorption cell fitted flush to the panel in a groove on its other side. This absorption cell has an inside thickness of 1 cm., so that the red light passes through a 1-cm. layer of liquid when the cell is filled.

To take a reading, the absorption cell is first filled with distilled water and the light switched on. The intensity of the light on the face of the photoelectric cell is rapidly adjusted by means of the rheostat so that the needle of the microammeter swings to exactly 200 microamperes, the maximum of the meter. Speed is essential in doing this, since even a small lamp is sufficient to "run down" the battery enough to cause an appreciable change in the light intensity of the bulb. Although this change is not visible to the eye, it is apparent upon the dial of the meter. When the light intensity has been satisfactorily adjusted, so that the needle of the meter will swing back to the maximum each time the light is switched on, the light is turned off and the absorption bottle filled with the water solution of potassium chlorophyllin to be measured. The light is switched on, and the decrease in the light intensity transmitted, due to the absorption of light by chlorophyll and measured by the microammeter, is observed. If purely comparative results are desired, the readings obtained will give an accurate means of comparison,—the smaller figures representing the greater absorption and hence the greater concentration of chlorophyll.

The method of preparation of the chlorophyll solutions previous to their quantitative determination is essentially that of WILLSTÄTTER and STOLL

(4), with more recent modifications suggested by SCHERTZ (3) and MORROW (1). Since several slight alterations in procedure have been made, the method is briefly outlined as follows:

The leaves are cut as free of petioles as possible, and their fresh green weight determined. They are then dried in an electric oven at 37° C., pulverized in a mortar, and the powder further dried to expel any remaining moisture. The time required for complete drying varies with the thickness of the leaves,—in most cases three or four days will suffice. The dry weight is then taken and the percentage of solid matter determined. One-half gm. of the dry leaf powder, which amount requires less solvent and is more completely extracted in a shorter time than a larger amount, is placed in a Soxhlet siphon extractor, and continuously extracted for 24 hours with a 1:1 mixture of ether and acetone. The solvent flask is immersed in water

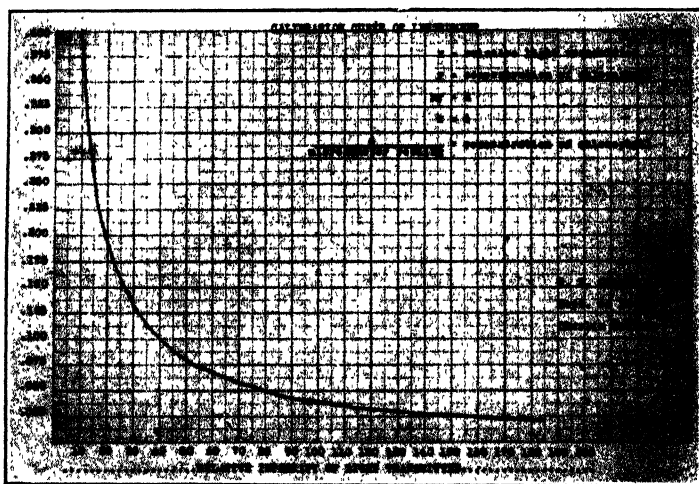


FIG. 3. Calibration curve of instrument.

kept at a temperature not exceeding 50° C. by means of an electric hot-plate. The solution is then placed in a separatory funnel, the acetone washed out with water, the anthocyanins and flavones with 1 per cent. sodium carbonate solution, and the ether solution washed twice more with water. The ether solution containing chlorophyll (alpha and beta) carotene and xanthophyll is then treated with 10 cc. of methyl alcohol saturated with KOH, and placed on ice for 24 hours. The potassium chlorophyllin separates out from the ether solution, leaving a clear golden-yellow liquid which contains the carotene and xanthophyll. By washing with water, the two layers are separated; the potassium chlorophyllin solution is washed *once* with ether and made up to 100 cc., at which point in the procedure it is ready for measurement in the instrument.

To calibrate the instrument so that the meter readings will be an actual indication of the amount of chlorophyll present in a solution, a series of solutions of known concentrations are made up from chemically pure chlorophyll. The chlorophyll is dried in a vacuum desiccator over sulphuric acid for a week, and ten accurate weighings made. When dry the chlorophyll is dissolved in ether, converted into its potassium salt by saponification with methyl KOH, and 40 different concentrations made up by dilution. The actual curve is constructed from the 40 readings obtained with these solutions in the instrument, with the x axis representing the microammeter readings (relative light transmitted) and the y axis the concentrations of chlorophyll in grams per 100 cc. This curve is found to be an hyperbola, with the equation  $xy = k$ .

The constant k for the 40 readings is averaged, and from the data already obtained in constructing the actual curve, an ideal curve is drawn. The calibration curve of the writer's instrument (fig. 3) differed only slightly from the actual curve first obtained. In this instrument, the constant k is found to be 4, so that

$ky = 4$  is the equation of the hyperbola, or

$$\frac{4}{\text{microammeter reading}} = \text{concentration of chlorophyll in grams per 100 cc.}$$

It must be emphasized that it will be necessary for each investigator to calibrate his own instrument and find his own ideal curve and equation, since no two photoelectric cells are exactly alike in their sensitivity to different wave lengths of light, or to different intensities of the same wave length. The purity of the chlorophyll samples used in calibration will also affect the constant k. In any case, however, the calibration curve will be an hyperbola, which approaches the x and y axes asymptotically, but the eccentricity depends on the constant k, and will vary according to the cell and the purity of the chlorophyll used in calibration.

Since 0.5 gm. of dry leaf powder has been used for extraction, the amount determined in the instrument is multiplied by two, which gives the amount of chlorophyll in 1 gm. of leaf powder. To refer this figure back to the green leaf material, it is multiplied by the percentage of solids in the green leaf, which has been determined when the leaves were dried. By dilution of the sample being determined, averaging the results after making allowance for the dilution will give a much more accurate figure than a single determination on each sample.

The instrument is not limited in its use to the quantitative determination of chlorophyll. It may be employed to match colors of dyes and to replace colorimetric methods now in use in many quantitative determinations. It can also be used to make certain *qualitative* color reactions *quantitative*, by employing light filters whose range corresponds to the strongest

absorption bands of the reaction product, and calibrating with solutions of known concentrations.

The writer wishes to acknowledge the helpful criticisms and suggestions of Dr. F. G. TUCKER and Mr. JAMES SNODGRASS of the Department of Physics of Oberlin College, and to express his appreciation to Professor F. O. GROVER for material aid in developing the apparatus.

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# GASOMETRIC METHOD OF ESTIMATING OXIDASE ACTIVITY

R. N. JEFFREY AND W. V. CRUESS

In the study of biological oxidations, two criteria are used as indications of oxidase activity. One of these is the oxidation-reduction potential of the system concerned, determined as described by CLARK (3) and others. The other is the rate of oxidation measured by one of several methods. In our particular studies on the oxidase systems of various fruits, measurement of rate is much more useful than measurement of oxidation-reduction potential.

## Rate measurements

Four important methods of measuring the rate of enzymic oxidation have been reported in the literature. These are the (1) colorimetric, (2) titrimetric, (3) electrometric, and (4) manometric methods.

If fruit oxidase is at all specific, it is very probable that the rate at which it oxidizes an added indicator is different from that at which it oxidizes the naturally occurring substrate. It is also possible that different catalysts are involved in oxidation of the natural and the added substrates. There are other objectionable features also, such as interference of turbidity of the juice, and difficulty in duplicating tints in permanent color standards.

Several of the more important colorimetric methods are those of DYE (6), RÖHMANN and SPITZER (13), and WILLSTÄTTER and WEBER (17). Most colorimetric methods require the addition of  $H_2O_2$ . This addition alters natural conditions, and CRUESS and FONG (4) have shown that its concentration greatly affects the results obtained.

GUTHRIE (8) uses in a titrimetric technique a special substrate formed by the action of NaOH on glucose solution. HAEHN and STERN (9) have reported upon a method in which unchanged added tyrosin is determined at intervals.

In the electrometric method of STEARN and DAY (15), hydroquinone of the quinone-hydroquinone complex used with the quinhydrone electrode is oxidized by the oxidase, changing the ratio of quinone to hydroquinone and thus the E.M.F. of the quinhydrone electrode. The resulting drift in potential is taken as a measure of the rate of oxidation.

One of the earliest applications of the manometric method was made by FOA (7). The well known BARCROFT manometric apparatus and its modified form, the WARBURG (16) apparatus, are in very general use. DIXON and ELLIOTT (5) have recently still further modified the BARCROFT apparatus. The BUNZEL (1, 2) apparatus also makes use of the manometric principle (see also SENNHAUSER 14).

Practical objections to the manometric apparatus are its high cost and

the rather large high differential pressures developed. The volume of liquid used is also small, usually less than 5 cc.

### Volumetric apparatus used

In the apparatus devised by the writers, a number of the objections to the colorimetric, titrimetric, potentiometric, and manometric methods are obviated. It is volumetric instead of manometric; hence the internal pressure need not exceed 1 mm. of Hg compared with pressures occasionally exceeding 50 mm. of Hg in the manometric method. There is, therefore, little tendency for leakage of gas outward or inward. The volume of the reaction flask may be much larger than in the manometric method, and it is not necessary that its volume be known accurately, since change in volume at atmospheric pressure is measured. Ground glass connections are not necessary, and since relatively large volumes of  $O_2$  are absorbed during the course of an experiment, standard gas burettes may be used for measurement of the volume of gas absorbed. Also, since the volume of the flask and burette is much larger in proportion to the volume of substrate than is the case in the usual manometric apparatus, there is less danger of retardation of reaction rate because of  $O_2$  absorption and reduction of the partial pressure of  $O_2$ . In building the apparatus common and relatively inexpensive laboratory equipment may be used.

The constant temperature bath is  $18 \times 18 \times 8$  inches, and of galvanized sheet metal thermally insulated by asbestos. The water is brought to operating temperature by means of a 500-watt knife-type Cenco "lagless" heater, and is maintained at operating temperature by means of a similar 125-watt heater controlled by a mercury-filled electric regulator and small relay. The relay is operated by a 6-volt battery charged by a radio battery charger. It is possible to maintain the temperature within a range of  $0.02^\circ C$ .

Four 250-cc. Erlenmeyer flasks are held in position in the bath by clamps to two movable  $\frac{3}{8}$ -inch rods suspended above the tank. The rods are moved back and forth horizontally at the rate of 90 times a minute by means of a  $\frac{1}{4}$ -H. P. motor, geared down to the proper speed by wooden pulleys.

The four Erlenmeyer reaction flasks are fitted with rubber stoppers and connected to gas burettes outside the reaction chamber by means of heavy-walled, capillary glass tubing. The volume of the connecting capillary of each flask is less than 1 cc. A stop-cock connected to a small thistle tube fitted through the stopper of each flask is used to introduce added solutions and to equalize the pressure at the beginning of the run.

There is suspended inside each flask from the stopper a short wire holding a small glass cup in which is placed a small roll of filter paper, cut in

the form of a rosette at the top, and saturated with N/1 NaOH solution to absorb  $\text{CO}_2$  liberated during the experiment. With fruit juices, the volume of  $\text{CO}_2$  liberated is relatively large and must be removed in this manner in order to avoid serious error.

The four gas burettes are attached by clamps to the desk in front of the constant temperature bath. The top of each burette is slightly above the surface of the bath, in order that the connection to the reaction flask shall be as short as possible. The connecting capillary glass tubing is joined to the burette and to the flask by flexible rubber connections. The connecting capillary glass tubing is cut above the flask stopper, and the ends held together tightly by a flexible rubber connection; it is similarly connected to the gas burette. The rubber connections permit movement by the shaker mechanism. Each burette is surrounded by a Pyrex glass jacket such as that used on Liebig condensers. The jackets are connected in parallel to the constant temperature water bath and to a small rotary pump from a discarded automobile engine. The pump is operated by the same  $\frac{1}{4}$ -H. P. motor that operates the reaction-flask shaker device, the pump and motor shafts being directly connected.

The burettes are filled with distilled water and the lower ends connected by rubber tubing to a single leveling bottle. Pinch-cocks on each tube permit leveling of each burette individually by the one bottle.

Mercury is of such high density that a small difference in height in the leveling bottle and burette represents a relatively large volume of gas. Clear petroleum oil of low density (nujol) gave a poor meniscus because of distillation of moisture from the reaction flask. Distilled water previously allowed to stand in air several days was found to be very satisfactory.

In order to prevent growth of algae in the bath and in the water jackets of the burettes, a small amount of formaldehyde (about 1:1000) was added to the water in the bath.

### Substrate

While it would have been desirable to use the volumetric apparatus without addition of an oxidizable substance, it was found that oxygen absorption was too small and inconsistent in the absence of such addition. Thus, 50 cc. of the freshly expressed apple juice absorbed less than 1 cc. of  $\text{O}_2$  in 130 minutes. Peaches gave a similar result.

BUNZEL (1, 2), in his tests with the oxidase of potatoes and beet leaves, used pyrogallol as the substrate in his manometric apparatus. However, we found that the rate of absorption of  $\text{O}_2$  by the pyrogallol was much greater at pH 5.7 in the absence of fruit juice than at pH 6.0 in juice. Evidently the fruit juice inhibited rather than catalyzed the reaction. At pH 10.3, non-enzymic oxidation was extremely rapid. Various tests gave additional evidence that pyrogallol, while probably satisfactory for potato

juice, is not very satisfactory for fruit juice. However, it proved useful with asparagus, spinach, string bean, and pea juices.<sup>1</sup>

Several of the commonly used colorimetric oxidase indicators, among them benzidine, hydroquinone, and guaiacol, were found to cause no significant additional  $O_2$  absorption. Tannins and fruit coloring matter from several sources also were found of no value as substrates.

Since catechol tannins occur naturally in many fruits, and because ONSLOW (10, 11, 12) has reported that plant materials that give a positive test with guaiacum contain substances with a catechol grouping, this phenol was used in a number of trials with apple, apricot, avocado, olive, prune, peach, and pear juices. It was found to be a very satisfactory substrate. There was also only slight absorption of  $O_2$  in water and in the boiled juice in the presence of the catechol, and absorption in these media ceased or became very slow after 15 minutes.

The  $CO_2$  evolved by respiration of fresh fruit tissues and freshly expressed juice under the conditions of our tests was found to be appreciable, and it was evident that some means of absorbing this gas (such as that previously described) is necessary. The initial rate of absorption increased with increase in ratio of enzyme to juice but not in a strictly proportional manner.

Although the  $O_2$  absorbed at the close of an experiment varied with the quantity of catechol initially present, the variation was not strictly proportional to the concentration of catechol. Thus when the quantity of catechol in one flask was 20 times that in another, the  $O_2$  absorbed was less than twice that in the second. Possibly the reaction product inhibits enzymic action at higher concentrations of catechol. Two cc. of 5 per cent. catechol to 50 cc. of sample was found satisfactory.

Portions of apple juice were brought to various pH values ranging from pH 2.25 to 6.75 by addition of NaOH or of N/1 acid. The pH of the untreated juice was 4.0. The rates of gas absorption were determined at 25° C.

Reducing the pH value from 4.0 to 3.7 very greatly retarded  $O_2$  absorption, and at 2.25–3.0 it practically ceased, being approximately the same as in boiled juice. Increasing the pH to 5.4 and 6.75 greatly increased the absorption of  $O_2$ .

Alkalinity naturally greatly favored  $O_2$  absorption. The rate of absorption and the total amount of  $O_2$  absorbed were much greater at pH 9, 10.1, and 10.8 than at pH 4.5; but much of this absorption is undoubtedly due to non-enzymic oxidation, as absorption is nearly as great in boiled as in unheated juices.

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<sup>1</sup> In tests made by H. M. PANCOAST.

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# LEAF-WRINKLE, A NUTRITIONAL DISORDER OF SOY BEAN<sup>1</sup>

E. W. HOPKINS<sup>2</sup>

(WITH ONE FIGURE)

In the course of a study of the effects of light conditions upon nodule formation by soy beans, irregularity of development of the leaves formed after the plants were 3-4 weeks old was observed. This condition showed varying degrees of severity, from cases in which only a few plants were affected to others in which practically all plants were injured.

Injury occurs only in young leaves, appearing first in unopened leaves 5-10 mm. long. At first the hairs at the leaf tip become brown, followed by browning of the leaf tip, and later the edge of the leaf shows injury for perhaps a third of its length. The leaf does not die, but continues growth. The dead areas at the tip and adjacent sides prevent the blade from expanding and the leaf has a puckered appearance. Leaves which have opened and are 2-3 cm. long before the smallest leaves show injury will usually die at the edges, and will be nearly as wrinkled as the later leaves. Fully expanded leaves do not seem to be affected. In figure 1A are shown injured young leaves which have begun to expand, and in figure 1B, healthy young leaves.

Addition of boron as boric acid did not afford protection from injury to leaves subsequently formed after injury was first observed; nor could the suggestion that the symptoms of the disease resembled those of soy bean mosaic be confirmed. Injured leaves were rubbed on the upper surface of healthy leaves of various size without producing transmission of the injury. Evidence to be presented indicates that the injury is due to a nutritional disorder.

Injuries of similar nature produced by faulty mineral nutrition have been reported for plants other than soy beans (2, 3), so it seemed desirable to consider the possible rôle of the nutrient solutions in this case. The nutrient solution previously used was modified in several ways.

Manchu variety soy beans were planted out-of-doors in a bed of no. 3 quartz sand and transplanted after 6 days to 2-gal. pots of quartz sand. Until 16 days after planting, no nutrient solution was added to the pots, but the pots were set in shallow pans and watered daily with tap water. On the 16th day the pots were taken into a greenhouse and the nutrient treatments begun. A liter of nutrient solution was supplied daily to each pot, using the drip culture method (4).

<sup>1</sup> Contribution from the Hull Botanical Laboratory, University of Chicago.

<sup>2</sup> National Research Council Fellow.

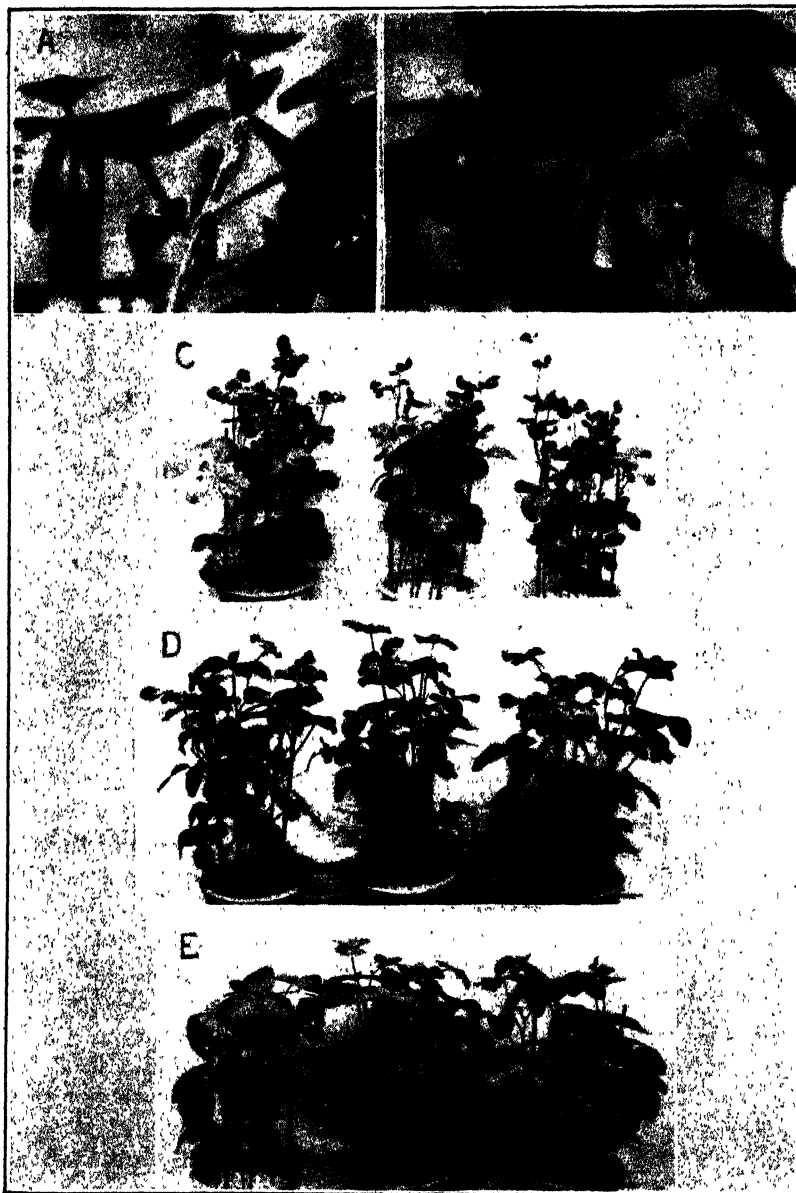


FIG. 1. A, B, young leaves of soy beans: A, leaf tips injured; B, leaf tips healthy. C, D, E, appearance of plants in three of the series: C, TIEDJENS and ROBBINS' solution + 30 mg. of  $\text{NO}_3\text{N}$  per liter as potassium nitrate (severe injury to leaves); D, TIEDJENS and ROBBINS' solution + 30 mg. of  $\text{NO}_3\text{N}$  per liter as calcium nitrate (very slight injury to leaves); E, BRYAN's solution + 30 mg. of  $\text{NO}_3\text{N}$  per liter as calcium nitrate (no injury to leaves).

Two basic nutrient solutions were used, BRYAN'S (1) solution to which calcium nitrate was added to give 30 mg. of  $\text{NO}_3\text{N}$  per liter of solution, and TIEDJENS and ROBBINS' (5) solution (series E, p. 12, modified by varying the nitrate) made up to contain 30 and 100 mg. per liter of solution of  $\text{NO}_3\text{N}$  as potassium nitrate or calcium nitrate. The basic formula of either solution was that for a nitrogen-free nutrient, and the nitrogen was added in varying amounts as indicated. The nutrient solutions had the following formulas:

BRYAN'S solution <sup>a</sup>		TIEDJENS and ROBBINS' solution	
$\text{K}_2\text{HPO}_4$ .....	0.00143	$\text{KH}_2\text{PO}_4$ .....	0.00633
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.00102	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.00237
$\text{CaCl}_2$ .....	0.00225	$\text{CaCl}_2$ .....	0.00146

The plants showed definite responses to the various nutrient treatments. In two of the treatments no injury to the young leaves was produced, and in the other three treatments injury occurred in differing degrees.

The plants receiving the BRYAN'S solution or the TIEDJENS and ROBBINS' solution plus 100 mg. of  $\text{NO}_3\text{N}$  as calcium nitrate were entirely free of affected leaf tips. Injury was most severe on the plants receiving nitrate as potassium nitrate. The group of plants given the TIEDJENS and ROBBINS' solution plus 30 mg. of  $\text{NO}_3\text{N}$  as potassium nitrate showed injured leaf tips 5 days after beginning nutrient treatment, and after 15 days few leaves were free from injury. The plants at the age of 41 days are shown in figure 1C. The TIEDJENS and ROBBINS' solution plus 100 mg. of  $\text{NO}_3\text{N}$  as potassium nitrate likewise produced dead leaf tips in 5 days, but after 15 days the number of injured leaves was considerably less than in the 30-mg. group.

Contrasted with the plants receiving potassium nitrate, those receiving calcium nitrate were relatively unaffected. On the plants given the TIEDJENS and ROBBINS' solution plus 30 mg. of  $\text{NO}_3\text{N}$  as calcium nitrate, a few injured leaves were evident 15 days after treatment began (fig. 1D). Figure 1E shows the plants which were given BRYAN'S solution. Table I indicates the composition of the nutrient solutions used (except for S and Cl) and the effect of these solutions on the leaf tips.

It is apparent from table I that the leaf-tip injury may not be ascribed to a particular cause on the basis of the present data. The difficulty does seem to arise from a complex relation of the various elements. If the nutrient solutions used in sets 1 and 2 be compared, it will be noted that if the N is increased there is less injury produced even though the amount of K present is considerably higher. Again, in sets 3 and 4, increase in N, in this case accompanied by an increase in Ca, results in no injury. In sets 4 and 5, where no injury occurred, the Ca/Mg ratio is about 4:1; and in

<sup>a</sup> The figures give the concentrations on the partial volume molar basis.

TABLE I

COMPOSITION OF THE NUTRIENT SOLUTIONS AND EFFECT ON PLANTS

TREATMENTS	K	Mg	P	Ca	N	RESULT ON PLANTS
	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	<i>p.p.m.</i>	
1. TIEDJENS and ROBBINS' + 30 mg. NO <sub>3</sub> N as KNO <sub>3</sub> . . . . .	289	57	196	58	30	Severe injury
2. TIEDJENS and ROBBINS' + 100 mg. NO <sub>3</sub> N as KNO <sub>3</sub> . . . . .	386	57	196	58	100	Less se- vere than in 1
3. TIEDJENS and ROBBINS' + 30 mg. NO <sub>3</sub> N as Ca(NO <sub>3</sub> ) <sub>2</sub> . . . . .	247	57	196	101	30	Slight injury
4. TIEDJENS and ROBBINS' + 100 mg. NO <sub>3</sub> N as Ca(NO <sub>3</sub> ) <sub>2</sub> . . . . .	247	57	196	201	100	No in- jury
5. BRYAN'S + 30 mg. NO <sub>3</sub> N as Ca(NO <sub>3</sub> ) <sub>2</sub> . . . . .	112	25	44	90	30	No in- jury

set 3, where slight injury was found, the ratio is about 2:1. The most severe injury was produced by the solutions in which the Ca/Mg ratio was about 1. However, the addition of nitrogen was effective to a considerable extent in preventing injury (set 2). The ratio of K/Ca follows an order the reverse of that of Ca to Mg.

The leaf injury to soy beans discussed here appears to result from the relation of K, Ca, Mg, and N in the nutrient solution used. The conclusion seems warranted that with the TIEDJENS and ROBBINS' solution a low nitrogen level tends to produce injury to the plants.

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## BRIEF PAPERS

### THE TROPOGRAPH AND ITS USE<sup>1</sup>

(WITH ONE FIGURE)

A few years ago, in studying some of the forces exerted by plants, the writer devised certain equipment with which he measured the force which the petiole of *Podophyllum peltatum* develops in its response to the stimulus of gravity, as it is kept from bending. This apparatus was described at that time and was given the name tropograph.<sup>2</sup>

In recent studies of tropisms, the tropograph has been completely rebuilt, with many changes, although the general principles have been maintained. Before presenting the results of these studies it was thought desirable to describe in detail the tropograph and to indicate some of its uses.

This equipment is illustrated in figure 1. A chemical balance (A) is

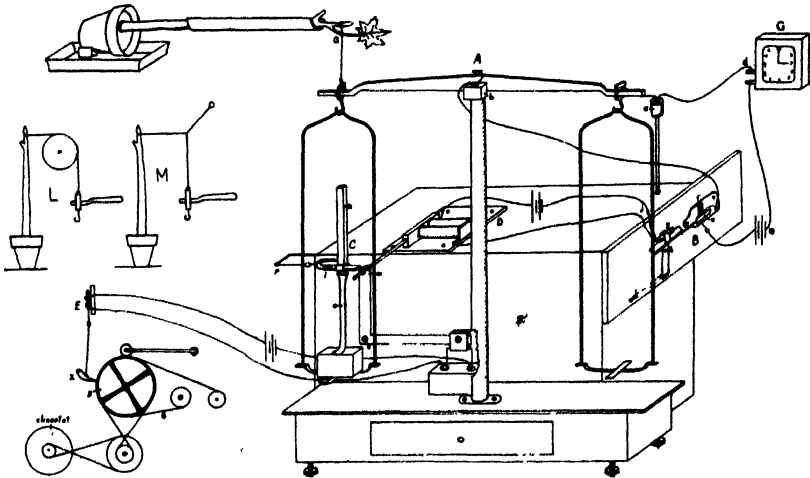


Fig. 1. The tropograph, an instrument for measuring the forces of tropic reactions.

used, and the end of the horizontal plant is connected by means of a thread or thin wire (a) to one end of the beam. To the other end of the balance is soldered a short piece of platinum wire which is curved so as to dip into a layer of oil covering the mercury in a mercury cup (c). By suitable weights in the pans of the balance, the latter is brought to equilibrium be-

<sup>1</sup> Contribution from the Department of Botany, University of Nebraska, no. 79.

<sup>2</sup> HIMMEL, W. J. A contribution to the biophysics of *Podophyllum* petioles. Bull. Torrey Bot. Club 54: 419-451. 1927.

fore the plant is attached. The end of the platinum wire and the mercury cup are so adjusted that, as the plant raises the opposite end of the beam only a fraction of a millimeter, the wire will come into contact with the mercury. An insulated wire connects the mercury with a clock (*G*) at *d*. The clock is so altered that it makes contact every minute between the wires leading into it at *d*. At the middle of the balance beam is also attached a short curved piece of platinum wire, by fastening it beneath the screw located at the top. This wire dips into a second mercury cup (*b*) so attached as not to interfere in the least with the freedom of movement of the balance. This wire may swing freely in the mercury as the beam moves. The other wire, connected with the mercury cup at *b*, connects with an electromagnet at *f*. An insulated wire connects this electromagnet with the clock through a set of two dry cells at *e*. When the plant, by pulling upward at *a*, makes contact at *c*, the clock completes the circuit and the electromagnet at *f* draws the armature (*x*) upward toward it.

To the left of this electromagnet (*f*) is a mercury switch (*g*) so mounted that it may swing freely on the pivot *h*. In operation this switch is nearly horizontal, but with the drop of mercury to the right. As the electromagnet operates, drawing the armature upward, the switch is tipped slightly the other way, causing the mercury drop to roll to the left, connecting the two wires at that end which lead away at *j*. A rubber band connecting the peg (*i*) and the base of the arm of the switch returns the switch to its original position.

The two wires at *j* connect with a second electromagnet (*D*) which is operated by a set of several dry cells. The electromagnet (*D*) operates a device (*C*) which drops steel balls, weighing approximately 1 gram each, into a container supported by the pan suspended from the left end of the beam of the balance and directly beneath the tip of the plant. The weights are located in tube (*k*), at the bottom of which is arranged a horizontal movable piece supporting the column of weights and attached to an extension of the armature of the electromagnet (*D*). This piece of metal slides in two lateral flanges at the bottom of the tube. When the mercury switch (*g*) closes the second circuit, the electromagnet (*D*) draws this piece at the bottom of the tube to the right and a steel ball falls through the hole in it at *l* which then is directly beneath tube (*k*). This movable base is curved so that the upper portion may move through a slit in the tube at the left and cut in between the two lowest balls, as the magnet (*D*) pulls it to the right. It thus supports the column of balls as the lowest one falls through the hole into tube *o* and on into the container on the balance pan. A rubber band attached at *p* helps pull the base back into its former position, thus permitting the column of weights to drop again on to the lower portion of the base so that another ball is in position to be released.

At *m* a light bar in the form of the letter **L** is attached to the extension of the arm of the electromagnet (*D*) and is pivoted at *n*. When the weight is dropped, the right end of this bar, which carries a wire shaped as an inverted **U**, is lowered so that the points of the wire dip into two mercury cups. This closes a third circuit in which a single dry cell and a third electromagnet are located, the latter operating an inking device at *z*. This inking device is continually describing a line on a strip of paper (*s*) which is carried around a revolving drum (*y*). When the electromagnet (*E*) operates, the inking device is drawn momentarily to one side, thus indicating when the weight is dropped. The drum is rotated on its axis in a clockwise direction by means of a clinostat to which it is belted through a couple of pulleys of different size, as indicated in the diagram, thus reducing the speed. On the top of the revolving drum (*y*) is a rubber roller which holds the paper against the drum. The rolls of paper used are the ordinary kind employed with adding machines. After the paper passes over the drum it is wrapped over a smaller cylinder which is rotated by a string wrapped about it at one end and which passes over an elevated pulley and is attached to a weight. This weight causes the cylinder to turn and keeps the paper taut.

Glycerin colored with a little gentian violet has been used for ink. This works very well, being just stiff enough to flow through the inking device at the proper speed and giving a clear, distinct mark. The inking device is a brass tube curved and tapered so that the opening at the tip is very small.

This equipment is equally suitable to the measurement of the force developed in response to light of various wave lengths and intensities, while the plant or plant part is not permitted to bend. When used in such studies, the plant is placed vertically and the thread connecting its tip with the balance beam is passed over a pulley as shown at *L* in the diagram. The tip of the vertical plant may also be connected to the end of the balance beam by means of a fine wire bent at right angles, and held in place by another wire connected at the apex of the angle in such manner that the pull by the plant in a horizontal direction will be equal to the upward pull on the balance. This is shown at *M* in the diagram.

The pot in which the plant has grown should stand or lie, as the case may be, in a tray as shown in the diagram, so that the plant may be supplied with water. This tray may be supported on a ring stand. The stem must also have some support for use as a fulcrum against which it may pull. The writer has used split glass tubing somewhat larger than the stem, and tied with linen thread around the hypocotyl or suitable internode of the stem. This may then be held in place by a burette clamp supported by a ring stand.

If less sturdy plants are being used, or if the force which the particular plant develops is not very great, a weight-dropping device adapted to small weights may be employed without altering any of the other parts of the tropograph.

By means of this apparatus the force which develops within the stem as it curves in response to the stimulus is automatically recorded. This force is equal at any minute to the sum of the weights in the container on the balance. The rate at which this force develops is thus recorded, as well as the maximum force which the stem is capable of exerting. The previous environment of the plant; that is, whether it has been in bright light or in the dark, will have a marked effect upon its response. This may be determined by the use of the tropograph. There seems to be a relationship between the food available within the plant and the force developed. The effect of the age of the plant, various gases, and chemicals upon the tropic force is also being investigated, as well as the relationship of the osmotic pressure of expressed sap to the tropic force as measured by the tropograph.

The principles employed here are not new, and have been used in the study of transpiration; but so far as the writer is aware they have not been employed in the study of tropic forces. It is hoped that these studies may contribute some information which will help in the more thorough understanding of the tropisms.—W. J. HIMMEL, *University of Nebraska, Lincoln, Nebraska*.

### RELATIVE INFLUENCE OF NITRATE AND AMMONIACAL NITROGEN UPON INTAKE OF CALCIUM BY TOBACCO PLANTS

Recent investigations in the Soils Department greenhouse at the Connecticut Agricultural Experiment Station at New Haven showed that when tobacco was grown in sand and water cultures with varying combinations of nitrate<sup>1</sup> and ammoniacal<sup>2</sup> nitrogen, and with equal amounts of the most essential elements, the calcium intake by the plant was increased in direct proportion to increments of nitrate nitrogen over ammoniacal nitrogen supplied in the nutrient solutions.

The sand cultures in which quartz sand was used were carried on in 2-gallon glazed earthenware jars. Two-quart glazed earthenware jars, with thickly paraffined three-ply wood covers with holes in the center, were used for the water cultures.

The nutrient solutions used contained equal amounts of the most essen-

<sup>1</sup> Calcium nitrate.

<sup>2</sup> Ammonium sulphate.

tial elements. The variable was the carrier of nitrogen. Five solutions with different proportions of nitrate and ammoniacal nitrogen were employed with the sand cultures. Two solutions, one containing all nitrate nitrogen and the other with all nitrogen in the ammoniacal form, were used with the water cultures.

Havana Seed tobacco plants about 1 inch high were set in the sand and water cultures. One and one-half liters of one-third strength nutrient solution were added to each culture. At the end of ten days the solutions were renewed, and the renewal process was repeated at the end of the next seven days with one-half strength solution. Full strength solutions were then used for two periods of seven days each, after which they were renewed every three and one-half days until maturity of the plants.

Three successive crops of tobacco were grown in duplicate. During the early period of the tests the plants made more growth with increased proportion of ammoniacal nitrogen in the cultures, but later the nitrate end became larger and surpassed the ammonia end at maturity. The ammoniacal solutions produced blue-green while the nitrate solutions produced yellow-green colored foliage. During sunny days when the greenhouse became abnormally warm, the plants wilted progressively with increase of ammoniacal nitrogen while the nitrate cultures remained turgid. The plants in the cultures that contained all the nitrogen in the form of ammonia were stunted in growth and had rather thick, leathery leaves.

When the plants from the sand cultures were harvested, the roots were carefully examined. A form of root-rot increased in severity toward the ammonia end. It was most evident where all the nitrogen supplied was in the ammonia form. The same was the case in water cultures. The all-nitrate supplied culture produced healthy white roots while the culture that

TABLE I

TOTAL CALCIUM OF TOBACCO PLANTS GROWN IN SAND AND WATER CULTURES

PROPORTIONS NO <sub>3</sub> N: NH <sub>3</sub> N		SAND CULTURES IN PER- CENTAGE OF Ca	WATER CULTURES IN PER- CENTAGE OF Ca
		%	%
All	0	3.483	1.794
$\frac{1}{2}$	$\frac{1}{2}$	2.368	.. ..
$\frac{1}{3}$	$\frac{1}{3}$	1.449	.... ..
$\frac{1}{4}$	$\frac{1}{4}$	0.983	.. .....
0	All	0.652	0.618

received only ammoniacal nitrogen produced badly discolored and rotted roots.

Total calcium analyses were made of the plant material from all the cultures for the three crops. An average of these analyses is shown in table I.

The analyses (table I) show definitely that an increased proportion of nitrate nitrogen over ammoniacal nitrogen resulted in a larger percentage of total calcium in the plant material.

The root-rot phase of the investigation is being intensively studied in cooperation with the Tobacco Substation at Windsor, Connecticut.—H. G. M. JACOBSON, *Agricultural Experiment Station, New Haven, Connecticut*, and T. R. SWANBACK, *Tobacco Substation, Windsor, Connecticut*.

## NOTES

**Atlantic City Meeting.**—Main features of the annual meeting were the incessant rain, the SACHS centennial celebration, and the annual dinner at which announcements of the award of the eighth life-membership and the third STEPHEN HALES prize were made. The annual dinner was also made the occasion of the reading of the second STEPHEN HALES address by Dr. W. W. GARNER.

The SACHS centennial was held in the Rose Room at the Traymore Hotel on December 28, 1932. A large gathering enjoyed the three addresses by Dr. D. H. CAMPBELL, of Stanford, Dr. R. H. TRUE, of Pennsylvania, and Dr. C. E. ALLEN, of Wisconsin.

In view of the small attendance at the banquet, it seems to be a mistake to place this function at the end of the meeting. It is suggested that the night preceding the Botanical Society dinner should be used by all other societies in the botanical field for annual dinners, so that all botanists may be free to attend the Botanical Society banquet. It would also be an advantage if tickets were prepared in advance of the meeting, and were placed on sale at the registration desk, and at all sessions of the Society.

**Stephen Hales Prize.**—The third award of the STEPHEN HALES prize was made to Dr. HUBERT BRADFORD VICKERY, of the Connecticut Agricultural Experiment Station, for his work on vegetable proteins. Dr. VICKERY was born at Yarmouth, Nova Scotia, February 28, 1893. He graduated with the B.S. and M.S. degrees from Dalhousie University, Halifax, in 1915 and 1918. He holds the Ph.D. degree from Yale in 1922. His early professional work was done in the high schools and normal college of Nova Scotia. During the last eleven years he has been research chemist at the Connecticut Station, and lecturer at Yale University since 1924. He has contributed a number of valuable papers on protein chemistry, and the constitution of the proteins of green plants.

**Summer Meeting.**—The meeting of the American Society of Plant Physiologists in June will be in connection with the A.A.A.S. World's Fair meeting. The meetings will be mainly joint meetings with other groups on June 20 and 21, with a special meeting of plant physiologists on Thursday morning, June 22. This special meeting will be a symposium on Radiation and Plant Life. The headquarters hotel for Section G and its affiliated societies is the Stevens Hotel. Early reservations should be made, as space will be limited by public demand. All meetings are being arranged to allow much freedom for visiting the exhibits at the Fair without missing important programs.

**Support of Publication.**—It has been necessary to defer a number of papers that were intended to appear in January and April, 1933, to later issues because of slow payment or non-payment of dues and renewal of subscriptions. It is impossible to use papers faster than our income allows, or to print a larger volume than our total finances will cover. This is a matter of importance to every member, and we hope that efforts will be made to maintain a stable income for *PLANT PHYSIOLOGY*. It is the only hope we have for continuance of the present generous-sized volume. The most serious consequence of reduced income is the necessity of holding all manuscripts for more than a year before they can be used. Papers intended for publication might as well be held for another year's work, and revised downward in size in addition.

**Plant Physiology Laboratory at Purdue University.**—The Department of Biology at Purdue University has had the good fortune to secure a new laboratory for plant physiology during the last year. Construction was effected during the summer and fall of 1932. The main laboratory is used for the introductory work in plant physiology. It is conveniently located adjacent to the physiology greenhouse, which is equipped with temperature control. A graduate laboratory provides facilities for graduate class work and research. A private research laboratory and office, and a centrally located stock room are also on the main floor.

An extensive basement has been constructed, and will be developed as funds become available. Plans for the basement include an oven room, a uniform temperature laboratory, a machine and work room, and adjacent light and dark rooms designed for plant growth experiments. An invitation is extended to plant physiologists to visit the laboratory when in Lafayette.  
—R. E. GIRTON.

**Chemical Methods Committee.**—This committee has under consideration the preparation of a supplementary report on chemical methods of analysis for plant materials. Dr. W. E. TOTTINGHAM, chairman of the committee would appreciate suggestions from members of the society as to matters which should be given attention in such a supplementary statement. He would be greatly assisted in this work if those who have had experience with new methods, or successes or failure with old methods would communicate with him at an early date.

**Errata.**—An unfortunate error was made in the notice accorded to *Anthokinetics*, by G. W. GOLDSMITH and A. L. HAFENRICHTER in the October, 1932, number of *PLANT PHYSIOLOGY*. On page 753, line 23, "heteranthous and ephemeral-heteranthous" should read "hemeranthous and ephemeral-hemeranthous."

On page 752, line 25, in the same number, for 400 read 4000. The editor regrets that these errors were not discovered in the proofs. Subscribers may find it useful to record the corrections where the errors occur to prevent future mistakes.

**Root Nodule Bacteria.**—A splendid monograph on root nodule bacteria and leguminous plants has been written by Dr. E. B. FRED, Dr. IRA L. BALDWIN, and ELIZABETH MCCOY. It is published by the University of Wisconsin as Studies in Science no. 5. It draws together the literature on this subject in masterly fashion. The approach is historical, and the background of present knowledge is skilfully arrayed. In the main part of the work the methods of isolation and study are presented, from which proceeds our knowledge of the morphology, life cycle, cultural and biochemical characteristics, longevity and species relationships of the organisms. The formation, histology and cytology of the nodules, mutual relationships between legumes and the bacteria, factors that influence nodulation, and natural and artificial inoculation are considered in later chapters. It is difficult to do justice to a work of this kind in a brief notice. There are 46 plates, a few charts, and almost a thousand citations. One can see that it will be of priceless value to other workers in this field, and that it will stand as a milestone of progress in the history of legume research. It may be obtained for \$3.00 from the University of Wisconsin, Madison, Wisconsin.

**Progress of Botany.**—The first volume of an annual series devoted to Fortschritte der Botanik has been published by Julius Springer, 23-24 Linkstrasse, Berlin W9. The editor is FRITZ VON WETTSTEIN, of Munich. The progress in morphology, systematic botany, physiology and ecology is delineated for sixteen subdivisions of these fields by sixteen collaborating authors. The publication of research has become so voluminous that summaries are essential to proper perspective. The value of such a work depends upon the breadth of view, experience, and judgment of the individual collaborators. This volume for the year 1931 is a good beginning. One can find in a compass of 15 to 20 pages the main advances made during the year in the particular fields of botany included. The sketches are very helpful, even if they are condensed. On the whole they represent a satisfactory picture of the advances made during the year. The price quoted for this volume is 18.8 R.M.

**Laboratory Manual of Plant Physiology.**—The second volume of Das kleine pflanzenphysiologische Praktikum by L. BRAUNER, has been published by G. Fischer, Jena. (See PLANT PHYSIOL. 5: 291-292. 1930, for notice of vol. I). The experiments outlined in this volume deal with physico-chemical phenomena of cells. There are 9 experiments on diffusion phenomena,

26 on osmotic action; 28 on permeability, and absorption, and 20 on imbibitional swelling. The outlines are clear, the directions adequate, and the experiments well chosen to demonstrate principles. There are 61 text figures, and a brief appendix with some useful data tables, and directions for some simple technical operations in preparing apparatus. The price in paper covers is 5.5 R.M., 7 R.M. in cloth binding. Orders may be sent direct to the publishers.

***Tribonema*.**—A brief monograph by EVA HAWLITSCHKA gives an account of this genus of heterokont algae (Die heterokonten Gattung *Tribonema*). The author gives the nomenclatural history of the genus, the present known geographic distribution, the results of artificial culturing in solutions and on agar media, methods of reproduction, vegetative (resting spores, aplanospores, zoospores) and sexual, cytological observations, and a discussion of the known species. It is a 36-page brochure with 18 figures. The price is 3 R.M. It can be ordered from the publisher, G. Fischer, Jena.

# PLANT PHYSIOLOGY

JULY, 1933

## COMPARATIVE RESPONSES OF LONG-DAY AND SHORT-DAY PLANTS TO RELATIVE LENGTH OF DAY AND NIGHT<sup>1</sup>

W. W. GARNER

The primary purpose of the present discussion is to contrast the behavior of the so-called long-day and short-day types of plants in terms of their response to differences in length of day and to consider the problem of satisfactorily classifying plants into these two groups. In the first paper by GARNER and ALLARD dealing with the effect of length of day on plant growth, published about 12 years ago, two broad facts were brought out with respect to initiation of sexual reproduction, namely: (1) that some plants are more sensitive than others to the length-of-day factor; (2) that of the more sensitive group some respond to relatively long days while others respond to relatively short days. Thus we have one group embracing what may be designated as the indeterminate type of plant and a second group which can be conveniently divided into the long-day type and the short-day type. This classification has proved helpful and it seems desirable that it be retained, at least until some better system is devised. It appears, however, that unless a better understanding can be reached as to the basis on which the classification rests, considerable confusion is likely to result.

In dealing with the action of length of day on plant growth, greatest interest naturally attaches to its formative effects, and in the writer's own studies the action of the light period in initiating or suppressing sexual reproduction has been stressed. It is on these effects that our classification of plants into long-day and short-day groups is based. In a recent general review of photoperiodism in plants, SCHICK<sup>2</sup> suggests that for those plants in which tuber formation is of importance, this reaction to the light period rather than flower formation be used as a basis for classification, and this

<sup>1</sup> Second STEPHEN HALES address; read before the American Society of Plant Physiologists at Atlantic City, December 30, 1932.

<sup>2</sup> SCHICK, R. Photoperiodismus. *Der Züchter* 4: 122-135. 1932.

proposal seems logical. On the other hand, some recent investigators have grouped their experimental material into long-day and short-day types on the basis of the growth rate under different day lengths. Obviously this represents an entirely different system of classification. Again, some investigators in this field have shown an inclination to apply the designation "long-day plants" to experimental material which has been merely exposed to a long-day treatment without regard to the response obtained, and, likewise, plants exposed experimentally to a short day would be designated as "short-day plants."

Assuming that initiation of processes of reproduction is accepted as the basis for classification, it might seem at first thought that long-day plants could be defined simply as those which respond to long days (or even continuous illumination) and short-day plants as those responding to short days. We should then be confronted, however, with the problem of determining what constitutes a long day or a short day. For example, the equatorial or equinoctial day length of 12 hours cannot be regarded as furnishing a satisfactory dividing line, for this would throw into the long-day group a very large proportion of the plants which on the basis of their photoperiodic responses could be regarded as properly belonging only in the short-day group. Again, if the line of separation between short days and long days be moved up, say to a light period of 13 or 14 hours, the lower limits for flowering in many plants regarded as really belonging in the long-day group would fall below this period. The fact is that there is a range in day length from about 12 to 14 hours which is common ground for many plants in both groups, in that they are able to flower within this range even though in most cases it would not be optimal for either group. Because of its extreme simplicity (at least in principle), it may be that this plan of arbitrarily designating a particular day length as a basis for separating the long-day and short-day types will persist despite its obvious weaknesses. If so, perhaps a 14-hour day may well be adopted to supply the dividing line.

#### Critical length of day for flowering

It is believed that the problem of satisfactorily separating the two groups of plants may be more logically approached from a somewhat different angle. While it seems to have been the impression of some that in general the more sensitive plants require a particular daylight period for successful flowering, ordinarily this is not the case. It is true that there is a fairly definite optimum length of day for flowering, but generally speaking there is also a rather wide range in day length on either side of the optimum in which flowering takes place with more or less facility. Through these ranges, differences in time required for attainment of the flowering stage,

though appreciable, are not large. To take a concrete case, the Biloxi variety of soy beans, a late-flowering or short-day type, has been found to have an optimum day length for flowering of about 9 hours; but under favorable conditions it is capable of flowering under all lengths of day, ranging from 5 to 12 hours, with a maximum delay of only about 7 or 10 days as compared with results under the optimum length of day. While there may be a few exceptions, the great majority of plants of both the long-day and the short-day type show these characteristics. In contrast with these relations, however, there exist in the case of the more sensitive plants what may be called critical day lengths for flowering which have a much narrower range for any particular plant. Taking again the Biloxi soy bean, the critical daylight period may be considered as lying between 13 and 14 hours. Under a 13-hour daily light period the plant flowers after a delay of some 10 or 12 days as compared with optimum conditions of day length. With a daylight period of 14 hours the critical point has been passed and the plant tends to become strictly vegetative, flowering being delayed for a more or less indefinite time. Likewise garden balsam flowers readily with a day length of about 14 hours but is greatly delayed in flowering with a day length of  $13\frac{1}{2}$  hours, and with shorter day lengths it becomes strictly vegetative. In many species representing both the long-day and the short-day types, it has been found that under suitable conditions a variation in day length of not more than one hour (or even less) constitutes the critical range, on the two sides of which definite contrast in response is obtained. On the one side the plant flowers readily while on the other side it tends to remain in the vegetative stage. The important point in this connection is that the group of plants which we have been in the habit of classing as the long-day type flower only when exposed to day lengths in excess of the critical, while the short-day plants are able to flower only under shorter day lengths than the critical. In the present stage of our knowledge of the subject this would seem to furnish a simple and logical basis for differentiating between the two groups of plants. It is to be noted that this method of classification is independent of any particular relationship between the actual duration of the critical light periods for the two types of plants, and it is not necessary to determine what constitutes a long day or a short day. From this point of view the decisive factor is not the actual length of the critical light period but the type of the response to light periods on the two sides of the critical. Thus it is even possible for the critical light period of a long-day type to be shorter than that of a short-day plant, although in the more typical cases this will not hold true. In general, designation of the two groups as long-day and short-day types is still justified under this method of classification.

### **Classification into long-day and short-day types and the factor of latitude**

In applying the method of classification here proposed, it is necessary only to compare the character of the responses to a light period in excess of the critical and to one shorter than the critical. This is not necessarily the same, however, as merely determining the responses to what would be considered relatively long and short day lengths at a given latitude. Of course, we do not know the extreme range in length of day needed to embrace the critical light periods of all plants, and there is less information as to the upper limits than as to the lower ranges required. Nevertheless the indications are that few plants have a critical daylight period of much less than 12 hours, and it seems safe to assume that a 10-hour day would provide an exposure well below the critical for nearly all plants. As to upper limits, it has been found that some plants have a critical daylight period as long as 17 hours, so that evidently a day length of at least 18 hours must be used to provide an exposure distinctly in excess of the critical. If it were not for the disturbing factor of change in maximum length of day with change of latitude, for purposes of orientation in a given locality it would be necessary only to compare the behavior of the test plant under a 10-hour day and the full summer-day length. If flowering be materially delayed under the short-day treatment, the plant is to be regarded as belonging to the long-day type, while it would be placed in the short-day group should the short-day treatment hasten flowering. In the event that the date of flowering remain substantially the same under the two treatments, the plant would have to be regarded as belonging to what may be termed the indeterminate group. But a test of this sort will ordinarily give a reasonably dependable conclusion only when marked differences in response under the two treatments are obtained.

In other words, if the test definitely indicates that the plant in question belongs either to the long-day or the short-day group, the result may be considered fairly conclusive. Unless the experiment is conducted at very high latitudes, however, a result indicating that the plant is not sensitive to the day-length factor is to be accepted as purely tentative. This is true because there can be no assurance that the maximum summer-day length exceeds the critical light period. In working in lower latitudes, however, this difficulty can easily be overcome by employing artificial light to prolong the illumination period to, say, 18 hours. This will certainly take care of the great majority of cases although it is possible that in rare instances additional intermediate light exposures will be required.

#### **Indeterminate group of plants**

This group may be regarded as embracing those plants which are capable of flowering more or less readily throughout the range in length

of day ordinarily encountered through the year and at different latitudes. In such plants the day-length effects are primarily of a quantitative rather than a qualitative nature. The size attained by the plant and the extent or amount of reproductive activities and other growth features may be materially influenced, so that such plants may have an optimum day length for flowering. The essential point is that this group has no critical light period with respect to flowering, and under suitable growing conditions its members tend to flower at practically all latitudes and at all seasons of the year. The plants of this group are designated as "indeterminate" for the reason that the length-of-day factor does not exercise a clearly defined determinative (formative) effect, while they also might be designated as intermediate in the sense that in some respects the plants resemble both the long-day and short-day types and serve as a sort of connecting link between the two.

In attempting to utilize the critical light-period relations as a basis for grouping plants with respect to the day-length response, the mistake is likely to be made of throwing into the indeterminate group many plants which do not properly belong there unless the precautions previously mentioned are applied. This is what will usually occur when all light exposures employed for purposes of orientation happen to fall on one side of the critical light period. It will be recalled that in general short-day plants are able to flower with more or less facility through a wide range in day lengths below the critical, while the same is true of long-day plants for day lengths above the critical. In each case the response within these ranges is such as to indicate that there is no critical light period and the plants simulate the behavior of the indeterminate group. These relationships are well illustrated by the responses to differences in day length of the early, medium, and late varieties of soy beans, the Mandarin, Peking, and Biloxi varieties respectively. In plantings made in the greenhouse at frequent intervals throughout the year at Washington, the Mandarin has not been materially affected as to time required for flowering by the seasonal change in length of day, which ranges from  $9\frac{1}{2}$  to 15 hours. This variety therefore shows the characteristics of the indeterminate group when grown at Washington. It has been shown, however, that the Mandarin has a definite critical light period of about 17 hours and at greater day lengths flowering ceases. Hence, at a latitude of about  $52^\circ$ , as in southern Canada, this variety together with the Peking and Biloxi varieties may be expected to show the characteristics of a typical short-day plant. In Washington, at latitude  $39^\circ$ , the Peking and Biloxi continue to behave as short-day plants. In northern Florida, with a maximum summer-day length of about 14 hours, the Peking presumably would cease to respond as a short-day plant and would simulate the behavior of the indeterminate group. Finally, the

Biloxi, because of its relatively short critical light period, apparently would continue to act as a short-day type considerably farther southward. The Mandarin soy bean illustrates the fact that according to the proposed system of classification a short-day plant may have a relatively very long critical light period.

It remains to point out that undoubtedly some plants are injured by a very long daily light period or by continuous illumination; and possibly there are some which are so sensitive to the factor of day length that the range in light periods favorable to flowering is rather narrow, so that day lengths both too long and too short would occur. If such plants exist they must have two critical light periods, one on either side of the optimum period for flowering. These plants obviously would have the characteristics of both the short-day and the long-day types under this method of classification, and it might become necessary to place them in a special group.

#### **Types of response in short-day and long-day plants to day lengths unfavorable for flowering**

It is characteristic of a large group of herbaceous plants that the pre-flowering period of vegetative activity is limited chiefly to a leaf-rosette stage, and normally elongation of the axis is more or less rapidly followed by flowering. In another group stem elongation is a prominent feature of the early stages of growth, and under some conditions the axis may continue to elongate for a prolonged period without the appearance of flowering. This last-named group seems to furnish typical short-day plants while the first group supplies typical long-day plants. Thus, when exposed to a day length in excess of the critical, there is in the short-day type pronounced, long-continued elongation of the stem without flowering, while exposure to a day length shorter than the critical quickly initiates reproductive activity. On the other hand, in the long-day plant, exposure to a length of day in excess of the critical results in elongation of the axis, which is promptly followed by flowering; while exposure to a length of day below the critical tends to limit development to a leaf-rosette stage. It would seem, therefore, that the plan of classification herein presented is more or less in accordance with lines of division seen in nature.

It is apparent that when grown at different latitudes or at different seasons of the year, plants of both the long-day and the short-day types as measured by their responses may frequently shift from their true positions into the indeterminate group. No instance has been found, however, in which changes in the daylight period are capable of causing a long-day plant to behave as a short-day type or a short-day plant to simulate the behavior of a long-day type. As already pointed out, it is possible that some highly sensitive plants will be found to combine the characteristics of both

types. Such plants typically would exhibit the leaf-rosette form under a length of day shorter than the lower critical light period; a day length between the two critical periods would produce a flowering stem; while day lengths in excess of the upper critical period would lead to extensive elongation of the axis without flowering.

### Interrelation of length of day and temperature

Normal response to the day-length factor of course will be dependent upon favorable conditions of temperature. While other factors of the environment may be operative in varying degrees, in nature the combined action of length of day and temperature appears to be of special importance. For present purposes it will be sufficient to consider this factor complex very briefly in its relation to adaptation of the long-day and short-day types to different latitudes and different seasons of the year. The length-of-day factor is essentially constant from year to year while the seasonal temperature usually is variable. Hence under ordinary circumstances temperature may be regarded as more commonly responsible for variations in plant growth and development from year to year, particularly with respect to such features as time of flowering. Nevertheless, through the seasons of the year there is usually a definite trend in temperature, and it may be noted that ordinarily the mean-temperature curve for a period of years in its course from month to month rather closely resembles the length-of-day curve. There is, however, one significant point of difference. There is a pronounced lag in temperature change as compared with change in length of day which, at Washington for example, amounts to some 3 or 4 weeks.

Beginning with the late winter season, the rapidly increasing length of day in regions beyond the tropics will tend to stimulate plant life to renewed growth, while in the main the lag in temperature rise will operate to retard growth. Initially the day length is short and hence favors flowering in the short-day type of plant but is unfavorable for flowering in the long-day type. With rapid increase in the daylight period with advance of season, successful flowering in the short-day type becomes essentially a question of whether the favorable range in day length will have passed before the temperature has arisen sufficiently to permit definite expression. Naturally this may depend at times upon whether the spring season happens to be relatively warm or cold. In the main it may be said that at high latitudes conditions are not favorable for successful spring flowering in short-day plants, although those which are capable of growing at relatively low temperatures have a distinct advantage over the less hardy forms. The situation is different with respect to long-day plants, for the increasing length of day with advance of the season is constantly becoming more favorable for flowering. The long days of late spring and early

summer of course are favorable for flowering only in the long-day group of plants. As the days begin to shorten in late summer, the conditions become particularly favorable for flowering in the short-day group. Here the distinct lag in fall of temperature as compared with decrease in day length is especially advantageous to this group. Successful flowering and fruiting will depend chiefly upon whether the plant is capable of responding to the day-length factor early enough for completion of the reproductive processes before the advent of cold weather. Of course, chances of successful reproduction are greater in those species which are capable of tolerating cool temperatures. Summarizing, it may be stated that with increasing latitude the conditions become less favorable for flowering in short-day plants, and particularly so in the spring because of the combined effect of day length and temperature. For those of the short-day group having a moderately high critical light period, more favorable conditions for flowering will occur in late summer or early fall. Long-day plants having a very high critical light period cannot well flower and fruit successfully except at high latitudes, and capacity to grow under cool temperatures is distinctly advantageous. On the whole, of course, the short-day group is best adapted to relatively low latitudes and the long-day group will be more successful in higher latitudes. In this connection the work of LUBIMENKO, MAXIMOW, and associates in Russia, TINCKER and associates in England, McCLELLAND in Puerto Rico, YOSHII, KONDO, and associates in Japan, SCHICK in Germany, and others seems to lend support to this theory that the length-of-day factor plays an important rôle in the natural distribution of plant species and in crop-plant adaptation.

### Effects of abnormal light periods

One of the most interesting observations on the length-of-day effect, which was made in the early stages of these investigations, is that when reduction in the number of hours of light received by the plant is accomplished by midday darkening, the striking effects on flowering obtained by excluding the early morning or late afternoon light are no longer seen. It has been demonstrated that this result involves primarily the light period and not changes in intensity or spectral composition of the illumination. The midday darkening does not initiate flowering in the short-day type, and usually it does not materially interfere with reproductive processes in the long-day type, even when the period of darkening is long enough to impair the general nutrition of the plant. Evidently the net result of this method of reducing the daily number of hours of illumination is to favor vegetative activity in short-day plants and to favor reproductive activity in long-day plants. In a sense the effects of the usual short-day and long-day treatments are reversed. Although the number of hours of daily illumination

totals only that constituting a day of 9 or 10 hours, the effects on both groups of plants are those of a long day.

In this treatment the plants are exposed to the normal night period of darkness and to a midday period of darkness, with short periods of illumination in the forenoon and afternoon, so that the light relations are rather complex. The same results, however, have been obtained consistently when long-day and short-day plants were exposed to short, equal alternations of light and darkness obtained by use of electric light. With natural illumination the condition of equal day and night is not far removed from the optimum for initiation of flowering in most plants of the short-day type, but this 12-hour day is more or less unfavorable for flowering in the long-day group. Similar relations have been found to apply when artificial illumination is used. On the other hand, when the equal alternations of light and darkness were reduced to 6 hours or 4 hours, flowering was hastened in long-day plants and delayed in short-day plants. As the periods of light and darkness were further shortened these results were accentuated. Thus all short alternations of light and darkness, extending down to those as short as 5 seconds, proved to be unfavorable for flowering in the short-day group but distinctly favorable in the long-day type. These relations persisted even though in some of the treatments the nutrition of the plant was seriously disturbed. It is evident that here, as in the case of midday darkening, exposure to short alternations of light and darkness produces results in both groups of plants similar to those obtained with a long day, so far as relates to reproductive activity. As regards vegetative activity and general nutrition conditions, no essential difference was observed between the two groups of plants. As the alternations of light and darkness were progressively shortened, increasing evidence of impaired nutrition, including chlorosis, attenuation, and decreased growth, culminated in alternations of about one minute; but with further shortening of the alternations there was a rapid return to approximately normal nutrition and growth. It is evident, therefore, that the effects on reproductive activity were dependent primarily on whether the long-day or short-day type of plant was involved; but effects on nutrition and growth were conditioned chiefly by the specific alternation of light and darkness rather than by the type of plant.

Summarizing the discussion, it will be seen that while development of the contrasted characteristics of the two groups of plants which have been classed as long-day and short-day is based almost entirely on their observed responses to differences in the light period, this mode of classification seems to be a more or less natural one. Certain contrasts in growth characteristics, together with the marked differences with respect to reproductive activity in relation to day length manifested by the two groups,

are in line with their general behavior in nature as well as with their comparative regional and seasonal growth characteristics. In their responses to abnormal light periods the two types also show definite contrast, so far as concerns initiation of reproductive activity, although apparently this does not hold true with respect to maintenance of the general nutrition of the plant. However, any adequate interpretation of the exact significance of these contrasted responses must be left to the future. For those plants which are more sensitive to the length-of-day factor, there exists a fairly definite critical light period which constitutes the dividing line between day lengths favorable to flowering and fruiting and those tending to produce a purely vegetative type of activity. In the short-day group flowering is initiated by day lengths shorter than the critical, and in the long-day group flowering is initiated by day lengths in excess of the critical. Commonly among annuals and herbaceous perennials the alternative vegetative stage of the short-day type is characterized by indeterminate elongation of the axis, while a prominent feature of the vegetative stage in the long-day type is embodied in a leaf-rosette form of growth without significant stem elongation. To determine the classification of a given plant it will usually suffice to observe the nature of the response to a day length less than, and to one greater than the critical light period. The essential characteristic of the less sensitive or indeterminate group of plants is that they possess no clearly defined critical light period.

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# EFFECTS OF STARVATION ON DISTRIBUTION OF MINERAL NUTRIENTS IN FRENCH PRUNE TREES GROWN IN CULTURE SOLUTIONS

H. L. COLBY

(WITH FIFTEEN FIGURES)

## Introduction

Leaf samples were taken for analysis, from the entire group of French prune trees starved for various elements (2), at three dates during 1930, the second year of starvation. The first collections were on May 15, the second on July 25, and the third and last on November 5. By the time of the last collection, several groups of trees (-Ca, -P, -N) had dropped all of their leaves, and therefore no analyses are reported for leaves of these groups, and for this date.

In most respects, the second year analyses (tables I, II; figs. 1, 2) agree with the analyses of the leaves taken at the close of the 1929 season. Leaves from -K cultures were consistently high (on dry-weight basis) in N and  $P_2O_5$  throughout the entire season of 1930. These leaves were about 30 per cent. above normal in CaO and MgO, but showed only 31 per cent. of normal  $K_2O$  on November 5.

The CaO content of leaves starved for *calcium* started at 51 per cent. of normal (on May 15), and fell to 38.6 per cent. normal by July 25. They were low in N and in ash, and high in MgO and  $K_2O$  throughout the season.

Leaves starved for *magnesium* showed a MgO content of 0.08 per cent. (24.5 per cent. of normal) on May 15, the MgO content remaining almost constant throughout the season. These leaves were extremely low in CaO; even lower than the CaO content of -Ca leaves on July 25, etc. The N content was very high throughout the season, being 3.85 per cent. of the dry weight on November 5 (which figure represents N in a third cycle of young leaves, the only foliage these trees had at the time).  $P_2O_5$  and ash content were also above normal;  $K_2O$  content fell off sharply from the early season to November 5, when only 0.705 per cent. (dry weight) was present.

Leaves starved for *phosphate* showed a low N content on May 15, and only 0.09 per cent.  $P_2O_5$  (19.3 per cent. of normal);  $P_2O_5$  was slightly higher by July 25. The leaves were high in MgO and rather low in CaO throughout the season.

Leaves starved for *sulphur* showed (May 15) 0.068 per cent. (dry weight)  $SO_3$ , which was 10.0 per cent. of the normal content. The  $SO_3$  rose slightly by July 25 and then fell back to 0.08 per cent. on November 5.

TABLE I

## STARVATION SERIES

COMPOSITION AS PERCENTAGE OF DRY WEIGHT OF GROUPS OF STARVED LEAVES (FRENCH PRUNE TREES, SAMPLES TAKEN DURING SECOND YEAR OF STARVATION)

1: LEAVES COLLECTED MAY 15; 2: JULY 25; 3: NOVEMBER 5

TREAT- MENT	N			ASH			P <sub>2</sub> O <sub>5</sub>			CaO			MgO			K <sub>2</sub> O			SO <sub>2</sub>		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
-K .....	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
-K + Na ..	3.5	2.8	2.7	6.83	8.4	11.0	0.67	0.66	0.66	1.69	2.33	3.16	0.70	0.97	0.349	0.55	0.647	0.507	—	—	—
-Ca .....	3.46	2.69	2.56	5.87	7.2	9.57	0.65	0.63	0.60	1.42	2.02	3.03	0.56	0.61	0.558	0.42	0.647	0.447	—	—	—
-Mg .....	2.79	1.74	—	6.17	8.9	—	0.46	0.36	—	0.46	0.57	—	0.71	0.64	—	1.52	2.19	—	—	—	—
-PO <sub>4</sub> .....	3.27	3.24	3.85	9.33	9.2	10.8	0.57	0.45	0.399	0.55	0.38	0.506	0.08	0.081	0.084	2.43	1.58	0.706	—	—	—
-SO <sub>4</sub> .....	2.75	2.63	—	6.66	10.9	—	0.09	0.11	—	1.06	1.29	—	0.53	0.69	—	1.60	1.16	—	—	—	—
-N .....	4.10	2.21	1.62	7.66	9.5	12.2	0.65	0.33	0.24	0.81	1.51	2.23	0.39	0.42	0.391	1.40	1.46	1.71	0.068	0.132	0.08
-N .....	1.61	1.26	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Complete or AZ	3.40	2.53	2.04	8.30	10.3	15.9	0.47	0.39	0.33	0.90	1.50	2.47	0.33	0.43	0.43	1.42	1.05	1.66	0.693	0.810	0.952

\* July 25 sample of -PO<sub>4</sub> leaves consisted of fallen ones.

**TABLE II**  
**STARVATION SERIES**  
**COMPOSITION AS PERCENTAGE OF ASH OF GROUPS OF STARVED LEAVES, SAMPLES TAKEN DURING SECOND YEAR OF STARVATION**  
**1: SAMPLE COLLECTED MAY 15; 2: JULY 25; 3: NOVEMBER 5**

TREAT- MENT	N			ASH			P <sub>2</sub> O <sub>5</sub>			CaO			MgO			K <sub>2</sub> O			SO <sub>2</sub>		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
-K	3.5	2.8	2.7	6.83	8.4	11.0	9.8	7.8	6.0	24.7	27.1	28.7	10.2	11.6	3.16	8.1	6.74	4.6	—	—	—
-K + Na	3.46	2.69	2.56	5.37	7.2	9.57	11.1	8.7	6.2	24.1	27.5	30.4	9.52	8.4	5.8	7.15	7.90	4.67	—	—	—
-Ca	2.79	1.74	—	6.17	8.9	—	7.5	4.0	—	7.4	6.4	—	12.0	7.2	—	24.0	24.8	—	—	—	—
-Mg	3.27	3.24	3.85	9.33	9.2	10.8	6.1	4.9	3.7	8.6	4.2	4.6	0.830	0.92	0.77	25.1	17.4	6.52	—	—	—
-PO <sub>4</sub> *	2.75	2.63	—	6.66	10.9	—	1.35	1.02	—	16.0	11.8	—	8.00	6.3	—	24.0	10.6	—	—	—	—
-SO <sub>2</sub>	4.10	2.21	1.62	7.66	9.5	12.2	8.5	3.47	4.9	10.6	14.5	18.3	5.1	4.5	3.2	18.2	15.6	14.0	0.88	1.39	0.65
Complete or AZ	3.40	2.53	2.04	8.30	10.3	15.9	5.6	3.78	2.07	12.1	15.2	15.7	4.2	4.4	2.7	17.1	10.2	10.5	8.3	7.8	6.06

\* July 25 sample of -P leaves consisted of fallen ones.

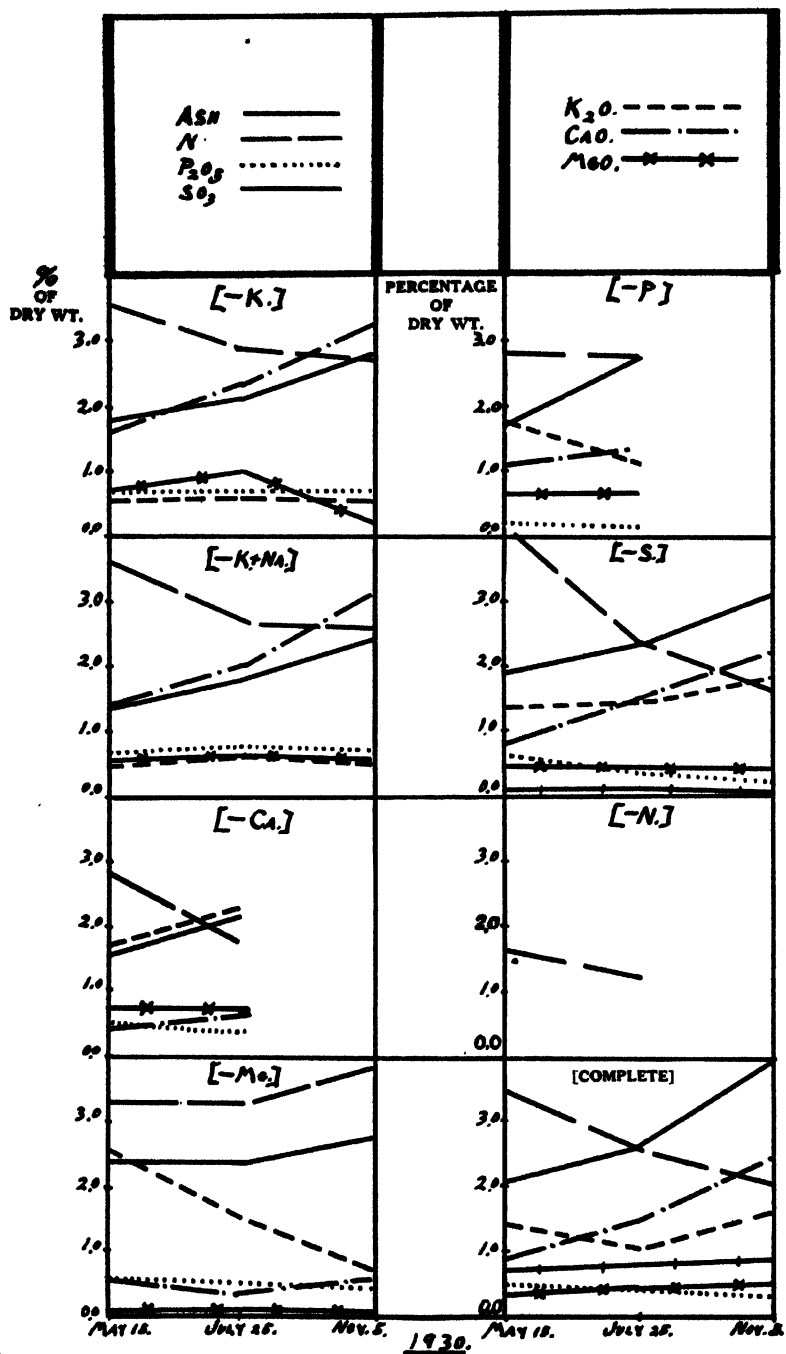


FIG. 1. Seasonal variation in the mineral composition of groups of French prune leaves started for various elements for the two-year period 1929-1930; samples taken on three dates during the second year of starvation.

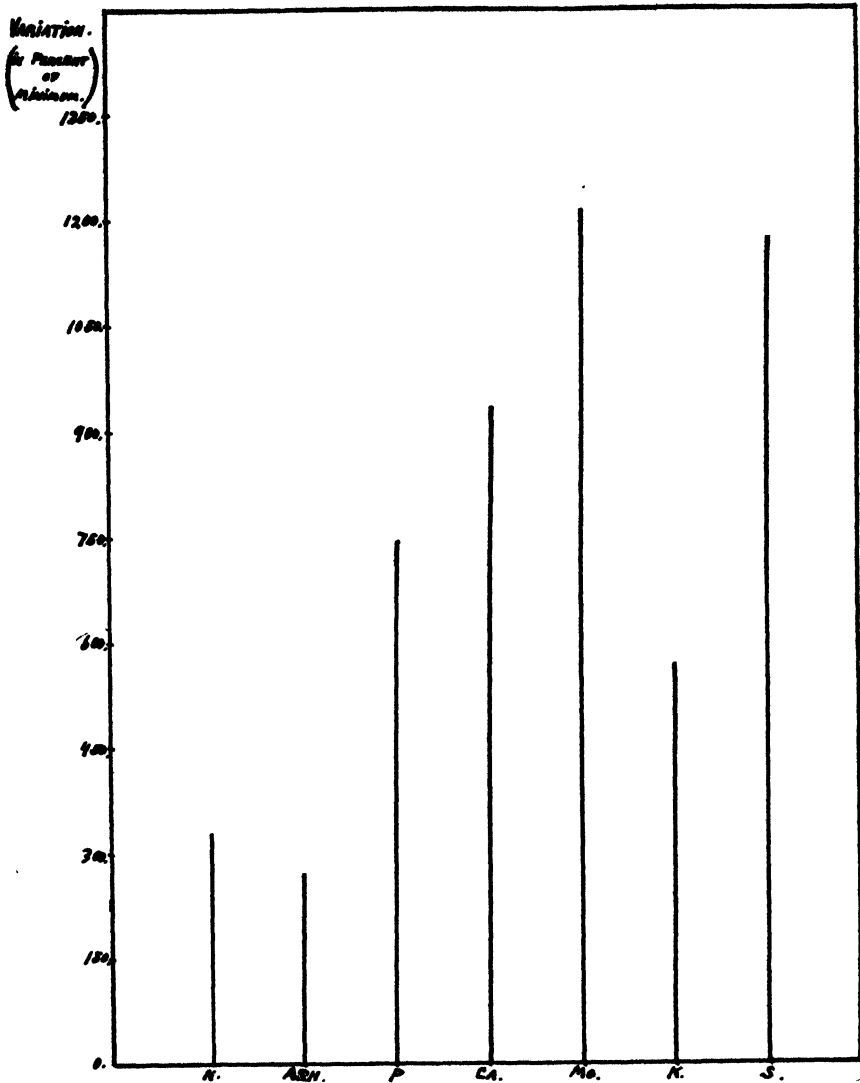


FIG. 2. Maximum variability of the mineral constituents (on percentage dry weight basis, the minimum in each case being taken as equal to 100 per cent.), obtained in starvation treatments, with French prune tree leaves taken in the second year of starvation.

The N content of -S leaves on May 15 was 4.7 per cent., the extreme upper limit of N content recorded anywhere in the experiment. The N fell rapidly to a low level of 1.62 per cent. by November 5.

Leaves starved for *nitrogen* showed 1.61 per cent. N on May 15 and 1.26 per cent. on July 25. No ash analyses were made of this group.

"*Complete*" (AZ) leaves showed 3.4 per cent. N at the first collection; 2.53 per cent. on July 25; and 2.04 per cent. on November 5. These leaves appeared to be below normal in  $K_2O$  throughout the season, although K was always present in the culture solution. They were slightly low in CaO content; otherwise the analyses checked fairly well with well-grown orchard leaves of similar trees.

The *maximum* (in percentage based on the minimum as = 100) content of particular elements, on a dry-weight basis produced by starvation methods in these leaves, was:

		PER CENT.
For total N		325
" " Ash		270
" " $P_2O_5$		744
" " CaO		831
" " MgO		1212
" " $K_2O$		566
" " $SO_3$		1180

#### Inorganic composition of bark, wood, and root at end of two years' starvation

After the eighty French prune trees of the starvation experiment had completed two years of growth in culture solutions, the trees were harvested, washed, weighed, and separated into six divisions: young (one and two-year) roots; main (three-year) roots; trunk (three-year) wood; trunk bark; young (one and two-year) branch wood; and young branch bark. All tissues were dried, ground, and analyzed for N, Mg, Ca, P, K, and Fe. Nitrogen was obtained by the Kjeldahl method, potassium by the platonic chloride (gravimetric), calcium by the volumetric ( $KMnO_4$  titration), magnesium by precipitation with ammonium phosphate, phosphorus by the Denigés colorimetric, and iron by the thiocyanate method.

The five trees taken from the group at the start of the experiment were separated into three divisions: trunk bark, trunk wood, and root, the last corresponding to the main root section of the starved trees harvested two years later.

In the original tree tissue (at the start of the experiment) the ash content of the top trunk bark was 6.36 per cent. of the dry weight; the ash content of the wood was 0.8 per cent.; and in the root (bark plus wood) it was 2.0 per cent. The roots were very high in phosphorus; the calcium and potassium contents were about equal; and most of the iron stored in the tree was in the root. The "original" trees, then, were moderately well

TABLE III

MINERAL CONSTITUENTS IN ORIGINAL TREE TISSUES AT START OF EXPERIMENT; IN PERCENTAGE OF DRY WEIGHT

	ASH	NITROGEN	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	SO <sub>3</sub>	CaO	MgO	Fe <sub>2</sub> O <sub>3</sub>
	%	%	%	%	%	%	%	%
Top Trunk } Bark }	6.36	1.8	0.34	1.82	0.3	1.97	0.15	0.055
Top Trunk } Wood }	0.80	0.75	0.07	0.39	0.02	0.15	0.048	0.0014
Root	2.00	1.10	0.24	0.70	0.09	0.70	0.087	0.046

stocked with the more common elements necessary for growth. Table IIIA gives the results on the basis of dry-weight yield for the entire tree.

TABLE IIIA

YIELD PER TREE OF DRY WEIGHT, ASH, AND NITROGEN, AFTER TWO YEARS' TREATMENT (STARVATION SERIES)

TREE	DRY WEIGHT	TOTAL ASH PER TREE	TOTAL N
	gm.	gm.	gm.
Complete	348.2	10.80	5.050
-S	200.2	6.30	3.300
-P	131.5	4.01	1.150
-K + Na	119.2	3.44	1.390
-Ca	98.3	2.32	0.710
-N	95.8	2.54	0.340
-K	92.4	2.79	1.048
-Mg	91.1	3.45	0.854
Original	53.3	1.01	0.540

### Inorganic composition of trees, taking whole tree as a unit

It is interesting to note that the *Mg-starved* trees were lowest of the series in dry-weight yield, but ranked fourth in total ash per tree, and sixth in total nitrogen. Mg-starved tissues throughout the tree were found to be high in ash content (the highest of any group of trees).

Trees starved for *potassium* showed (tables IV-IX; figs. 3-10) low N (percentage dry weight, compared with that of check trees), slightly lower ash content, high P<sub>2</sub>O<sub>5</sub>, normal MgO, and CaO equal to twice that of check trees. K<sub>2</sub>O was equal to 40 per cent. of normal.

Similar composition was found in the -K + Na trees, but with somewhat higher N content, lower P<sub>2</sub>O<sub>5</sub>, and much lower ash and CaO content than

**TABLE IV**  
**TOTAL NITROGEN CONTENT AND ASH CONTENT AS PERCENTAGES OF DRY WEIGHT IN PARTS OF FRENCH PRUNE TREES**  
**AFTER TWO YEARS' STARVATION**

TREAT- MENT	LEAVES TAKEN JULY 25, 1930		BARK (1 AND 2 YEARS)		BARK TRUNK (3 YEARS)		WOOD (1 AND 2 YEARS)		WOOD TRUNK (3 YEARS)		ROOTS (1 AND 2 YEARS)		ROOTS MAIN (3 YEARS)	
	N	ASH	N	ASH	N	ASH	N	ASH	N	ASH	N	ASH	N	ASH
-K . . . . .	2.8	8.4	1.6	6.9	1.14	6.1	0.7	0.8	0.25	0.55	2.05	4.1	1.37	3.0
-K + Na . . . .	2.69	7.23	1.51	5.6	1.22	7.8	0.605	1.15	0.24	0.77	2.31	4.15	1.63	2.66
-Ca . . . . .	1.74	8.9	1.04	4.0	1.02	4.5	—	1.2	0.15	0.60	1.47	3.85	0.91	2.6
-Mg . . . . .	3.24	9.2	1.52	8.4	0.78	8.6	0.52	1.6	0.12	0.70	1.88	6.55	1.13	3.86
-NO <sub>3</sub> . . . . .	1.26		0.62	4.4	0.51	5.0	0.36	1.63	0.09	0.62	0.77	4.55	0.32	2.8
-PO <sub>4</sub> . . . . .	2.63	10.9	1.25	5.3	1.09	5.8	0.44	1.33	0.15	0.70	1.68	4.35	0.88	3.1
-SO <sub>4</sub> . . . . .	2.21	9.5	2.31	5.7	2.10	4.7	0.47	0.92	0.36	0.52	2.49	5.15	1.90	2.7
Complete . . . .	2.53	10.3	1.57	4.9	1.35	6.4	0.67	0.80	0.31	0.55	2.68	5.05	1.70	2.6
Distilled H <sub>2</sub> O			0.27	3.1	0.24	2.5					1.4	3.8	0.33	2.2

TABLE V  
P<sub>2</sub>O<sub>5</sub> AS PERCENTAGE OF ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PARTS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK (1 AND 2 YEARS)		BARK TRUNK		WOOD (1 AND 2 YEARS)		WOOD TRUNK		ROOT (1 AND 2 YEARS)		ROOT MAIN (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
-K	7.8	0.66	6.6	0.455	5.4	0.329	22.5	0.180	43.6	0.24	9.5	0.389	12.5	0.375
-K+Na	8.7	0.63	8.5	0.476	4.6	0.358	17.3	0.198	25.3	0.19	9.7	0.397	14.4	0.384
-Ca	4.0	0.36	11.7	0.468	6.6	0.297			21.0	0.127	9.6	0.364	10.4	0.270
-Mg	4.9	0.45	6.7	0.562	2.1	0.180	8.7	0.149	9.6	0.067	10.3	0.669	8.6	0.336
-NO <sub>3</sub>			8.8	0.388	4.4	0.220	9.2	0.139	20.5	0.127	12.4	0.558	7.7	0.213
-PO <sub>4</sub>	1.02	0.112	5.1	0.270	1.5	0.087	3.8	0.0494	6.4	0.045	2.8	0.120	3.3	0.101
-SO <sub>4</sub>	3.47	0.33	7.0	0.402	7.02	0.329	15.2	0.139	15.9	0.082	9.4	0.479	11.2	0.303
Complete	3.78	0.39	9.2	0.450	5.10	0.326	18.7	0.148	23.0	0.127	7.4	0.370	14.2	0.384
Distilled H <sub>2</sub> O			Top 12	Top 12	Top trunk									
			8.7	0.270	4.7	0.117					11.8	0.448	8.5	0.193

TABLE VI

K<sub>2</sub>O IN PERCENTAGE ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PORTIONS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK (1 AND 2 YEARS)		BARK TRUNK (3 YEARS)		WOOD (1 AND 2 YEARS)		WOOD TRUNK (3 YEARS)		ROOT (1 AND 2 YEARS)		ROOT MAIN OR TRUNK (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
- K . . . . .	% 6.74	% 0.647	% 8.2	% 0.565	% 9.8	% 0.597	% 15.6	% 0.125	% 11.8	% 0.066	% 8.8	% 0.360	% 8.8	% 0.264
- K + Na . . . . .	7.9	0.647	9.3	0.510	7.6	0.592	9.1	0.104	18.0	0.138	8.4	0.344	10.0	0.266
- Ca . . . . .	24.8	2.19	26.9	1.070	25.4	1.140	35.6	0.430	44.0	0.264	33.2	1.26	31.5	0.810
- Mg . . . . .	17.4*	1.58	16.1	1.356	17.2	1.460	22.8	0.364	47.8	0.333	22.8	1.48	20.0	0.772
- NO <sub>3</sub> . . . . .	.	.	21.5	0.946	23.7	1.180	14.4	0.234	33.6	0.208	26.3	1.16	22.9	0.641
- PO <sub>4</sub> . . . . .	10.6	1.16	19.0	1.000	24.4	1.410	17.0	0.226	30.0	0.210	19.3	0.829	17.4	0.539
- SO <sub>4</sub> . . . . .	15.6	1.46	21.7	1.020	17.4	0.990	17.3	0.159	30.1	0.156	12.0	0.612	24.2	0.653
Complete . . . . .	10.2	1.05	20.0	0.980	17.2	1.100	28.1	0.225	30.0	0.165	18.4	0.920	22.6	0.587
Distilled H <sub>2</sub> O . . . . .	.	.	Top 12 16.1	0.499	Top trunk 16.4	0.410					13.3	0.505	13.2	0.288

TABLE VII

CaO IN PERCENTAGE OF ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PORTIONS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK TRUNK		WOOD (1 AND 2 YEARS)		WOOD TRUNK (3 YEARS)		ROOT (1 AND 2 YEARS)		ROOT MAIN (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
	%	%	%	%	%	%	%	%	%	%	%	%
-K .....	27.1	2.33	31.6	2.18	48.8	2.97	24.2	0.193	28.4	0.156	27.7	1.13
-K + Na .....	27.5	2.02	43.8	2.45	35.8	2.79	16.0	0.184	16.7	0.128	13.3	0.54
-Ca .....	6.4	0.57	6.6	0.26	17.3	0.78	9.4	0.113	19.2	0.115	4.4	0.167
-Mg .....	4.2	0.38	36.5	3.06	41.6	3.57	21.5	0.344	28.3	0.198	20.5	1.332
-NO <sub>3</sub> .....	...	...	23.6	1.148	28.0	1.40	10.6	0.173	28.8	0.178	4.0	0.183
-PO <sub>4</sub> .....	11.8	1.29	30.2	1.60	37.9	2.20	18.4	0.244	22.5	0.157	11.3	0.486
-SO <sub>4</sub> .....	14.5	1.51	31.1	1.49	39.8	2.19	19.4	0.178	28.1	0.146	9.3	0.474
Complete .....	15.2	1.50	25.0	1.22	29.7	1.90	14.3	0.114	26.0	0.143	6.7	0.335
Distilled H <sub>2</sub> O .....	...	...	Top 12		Top trunk		...	...	...	...	9.1	0.340
			15.2	0.480	15.6	0.380					13.7	0.310

TABLE VIII

MgO AS PERCENTAGE OF ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PORTIONS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK (1 AND 2 YEARS)		BARK TRUNK		WOOD (1 AND 2 YEARS)		WOOD TRUNK		ROOT (1 AND 2 YEARS)		ROOT MAIN (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
- K	11.6	0.97	4.4	0.303	5.9	0.359	4.0	0.032	2.1	0.0115	3.3	0.135	5.1	0.153
- K + Na	8.4	0.61	4.71	0.262	4.4	0.342	3.0	0.034	2.11	0.0161	4.8	0.196	5.7	0.148
- Ca	7.24	0.64	4.8	0.192	6.7	0.301	7.3	0.088	3.0	0.018	6.95	0.262	6.7	0.174
- Mg	0.92	0.081	1.2	0.100	0.87	0.074	1.6	0.025	1.32	0.009	0.96	0.062	2.0	0.076
- NO <sub>3</sub>	...	...	7.6	0.343	4.1	0.205	3.2	0.052	3.7	0.022	1.84	0.081	3.6	0.100
- PO <sub>4</sub>	6.3	0.697	5.7	0.302	4.5	0.261	2.6	0.034	2.4	0.016	3.40	0.146	3.7	0.114
- SO <sub>4</sub>	4.5	0.425	4.3	0.259	6.3	0.356	3.8	0.036	4.5	0.0234	4.40	0.224	5.5	0.148
Complete	4.4	0.43	4.2	0.205	4.2	0.268	3.7	0.029	3.8	0.0209	3.57	0.178	5.6	0.145
Distilled H <sub>2</sub> O	...	...	7.6	0.235	5.6	0.140	...	...	...	...	4.7	0.178	6.1	0.134

TABLE IX

Fe<sub>2</sub>O<sub>3</sub> AS PERCENTAGE OF ASH AND AS PERCENTAGE OF DRY WEIGHT IN VARIOUS PARTS OF FRENCH PRUNE TREES AFTER TWO YEARS' STARVATION

TREATMENT	LEAVES TAKEN JULY 25		BARK (1 AND 2 YEARS)		BARK TRUNK (3 YEARS)		WOOD (1 AND 2 YEARS)		WOOD TRUNK (3 YEARS)		ROOT (1 AND 2 YEARS)		ROOT MAIN (3 YEARS)	
	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.	ASH	DRY WT.
	%	%	%	%	%	%	%	%	%	%	%	%	%	%
- K	0.78	0.065	0.48	0.030	0.40	0.024	2.34	0.016	0.85	0.0047	8.8	0.360	3.2	0.096
- K + Na	1.20	0.086	0.68	0.038	0.49	0.038	1.90	0.021	1.02	0.0078	9.0	0.373	3.22	0.083
- Ca	1.40	0.126	1.091	0.046	0.89	0.040	0.66	0.007	0.78	0.0046	3.7	0.142	1.70	0.044
- Mg	2.40*	0.220	0.38	0.032	0.70	0.060	1.12	0.017	1.21	0.0084	6.7	0.435	1.51	0.058
- NO <sub>3</sub>	...	...	0.78	0.034	1.30	0.065	0.86	0.014	0.86	0.0053	3.4	0.156	1.02	0.028
- PO <sub>4</sub>	2.0	0.218	0.87	0.046	0.77	0.044	0.56	0.0073	0.87	0.0061	6.8	0.292	0.87	0.027
- SO <sub>4</sub>	1.2	0.114	0.62	0.024	0.43	0.029	1.90	0.018	1.37	0.0071	3.3	0.170	1.06	0.028
Complete	1.56	0.161	0.49	0.024	0.39	0.025	1.12	0.0088	1.24	0.0068	2.3	0.105	0.72	0.0187
Original tissue	...	...	...	...	0.85	0.055	...	...	0.175	0.0014	...	...	2.30	0.0460
Distilled H <sub>2</sub> O	...	...	0.98	0.031	0.77	0.019	...	...	...	...	0.61	0.023	0.80	0.018

**TABLE X**  
**DISTRIBUTION (AVERAGED) OF DRY WEIGHT AND MINERAL CONSTITUENTS**

TREATMENT	MATERIAL	DRY WEIGHT		TOTAL N		P <sub>2</sub> O <sub>5</sub>	
		GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL
		<i>gm.</i>	<i>%</i>	<i>gm.</i>	<i>%</i>	<i>gm.</i>	<i>%</i>
- K	Wood 1, 2	3.6	3.8	0.025	2.31	0.006	1.9
	Wood trunk	25.3	27.3	0.063	6.10	0.0607	19.7
	Bark 1, 2	6.7	7.3	0.107	10.1	0.0305	10.0
	Bark trunk	9.8	10.8	0.1117	10.6	0.0320	10.4
	Root 1, 2	14.4	15.5	0.2952	28.3	0.0559	18.2
	Root trunk	32.6	35.3	0.4466	42.6	0.1222	39.8
Total		92.4	100.0	1.0485	100.0	0.3073	100.0
Tree average				1.13%		0.33%	
- K + Na	Wood 1, 2	7.1	6.0	0.043	3.0	0.014	3.7
	Wood trunk	38.7	32.5	0.093	6.6	0.075	20.0
	Bark 1, 2	7.5	6.3	0.113	8.1	0.035	9.4
	Bark trunk	11.9	10.0	0.145	10.4	0.041	11.3
	Root 1, 2	17.7	14.8	0.408	29.4	0.070	18.7
	Root trunk	36.2	30.4	0.590	42.5	0.139	36.6
Total		119.2	100.0	1.392	100.0	0.3769	100.0
Tree average				1.17%		0.31%	
- Ca*	Wood 1, 2	4.6*	4.7	0.023	3.2	0.0068	2.8
	Wood trunk	30.2	30.7	0.0458	6.2	0.0383	16.1
	Bark 1, 2	5.1	5.5	0.053	7.4	0.0238	10.0
	Bark trunk	14.0	14.2	0.1428	20.0	0.0415	17.4
	Root 1, 2	8.0	8.2	0.1176	16.5	0.0291	12.3
	Root trunk	36.4	37.0	0.3312	46.6	0.0983	41.4
Total		98.3	100.0	0.7129	100.0	0.2378	100.0
Tree average				0.727%		0.24%	
- Mg	Wood 1, 2	5.5	6.04	0.0286	3.5	0.0076	2.9
	Wood trunk	29.5	32.4	0.0354	4.4	0.0197	7.7
	Bark 1, 2	7.8	8.6	0.1170	14.7	0.0438	17.1
	Bark trunk	8.3	9.1	0.0647	8.0	0.0149	5.8
	Root 1, 2	10.8	11.8	0.2030	27.5	0.0722	28.2
	Root trunk	29.2	32.1	0.3299	42.1	0.0981	38.3
Total		91.1	100.0	0.7786	100.0	0.2503	100.0
Tree average				0.854%		0.280%	

TABLE X

THROUGHOUT THE TREE (FRENCH PRUNE) AFTER TWO YEARS' TREATMENT

ASH		CaO		MgO		K <sub>2</sub> O		Fe <sub>2</sub> O <sub>3</sub>	
GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL
<i>gm.</i>	%	<i>gm.</i>	%	<i>gm.</i>	%	<i>gm.</i>	%	<i>gm.</i>	%
0.0288	1.0	0.0067	0.7	0.0011	0.9	0.0047	1.8	0.00057	0.6
0.1391	4.9	0.0394	4.3	0.0029	2.2	0.0167	6.5	0.00119	1.4
0.4623	16.5	0.1460	15.4	0.0203	16.4	0.0378	14.8	0.00280	3.1
0.5978	21.3	0.2910	30.8	0.0351	27.2	0.0585	22.9	0.00235	2.5
0.5904	21.1	0.1627	17.3	0.0194	15.1	0.0518	20.3	0.0518	57.4
0.9780	34.9	0.2973	31.5	0.0498	38.2	0.0860	33.7	0.0313	35.0
2.7964	100.0	0.9431	100.0	0.1286	100.0	0.2555	100.0	0.09000	100.0
3.02%		1.02%		0.13%		0.27%		0.098%	
0.0816	2.5	0.013	1.4	0.0024	1.5	0.0073	2.2	0.0015	1.4
0.297	8.4	0.049	5.6	0.0062	3.9	0.0530	16.2	0.0029	2.8
0.420	12.4	0.1837	20.7	0.0196	12.4	0.0380	11.6	0.0028	2.7
0.928	27.0	0.3320	37.5	0.0408	26.0	0.0704	21.8	0.0045	4.1
0.734	21.4	0.0964	10.9	0.0347	22.1	0.0608	18.6	0.0660	61.1
0.962	28.3	0.2099	23.9	0.0535	34.1	0.0963	29.6	0.0300	27.9
3.446	100.0	0.8845	100.0	0.1572	100.0	0.3264	100.0	0.1077	100.0
2.65%		0.74%		0.132%		0.27%		0.090%	
0.0556	2.3	0.00519	1.7	0.0040	2.7	0.0197	2.7	0.0003	0.8
0.1812	7.7	0.0347	11.8	0.0054	3.7	0.0797	11.2	0.00144	3.9
0.2065	8.8	0.01341	4.6	0.0097	6.8	0.0545	7.6	0.00224	6.0
0.6300	27.1	0.1089	37.3	0.0421	29.3	0.1596	22.4	0.0056	15.1
0.3080	13.3	0.0133	4.6	0.0209	14.7	0.1008	14.2	0.0113	30.7
0.9464	40.8	0.1172	40.0	0.0633	43.8	0.2970	41.9	0.0160	43.5
2.3277	100.0	0.2926	100.0	0.1454	100.0	0.7113	100.0	0.0368	100.0
2.37%		0.298%		0.14%		0.72%		0.037%	
0.088	2.5	0.0189	1.7	0.0013	2.9	0.020	2.7	0.00093	1.2
0.2065	5.9	0.0584	5.3	0.0025	5.5	0.098	13.4	0.00247	3.3
0.6552	18.9	0.2386	22.1	0.0078	16.5	0.105	14.3	0.00248	3.3
0.7138	20.7	0.2963	27.3	0.0061	13.2	0.1211	16.7	0.0049	6.4
0.6620	19.2	0.1427	13.1	0.0067	14.5	0.1598	21.9	0.0469	63.2
0.1271	32.0	0.3299	30.5	0.0222	47.4	0.2254	31.0	0.0169	22.6
3.4526	100.0	1.0848	100.0	0.0467	100.0	0.7298	100.0	0.0744	100.0
3.79%		1.19%		0.05%		0.80%		0.081%	

TABLE X (Continued)

DISTRIBUTION (AVERAGED) OF DRY WEIGHT AND MINERAL CONSTITUENTS

TREATMENT	MATERIAL	DRY WEIGHT		TOTAL N		P <sub>2</sub> O <sub>5</sub>	
		GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL
		gm.	%	gm.	%	gm.	%
- NO <sub>3</sub>	Wood 1, 2	0.8	0.83	0.0028	0.8	0.0012	0.5
	Wood trunk	31.7	33.10	0.0285	8.4	0.0402	16.5
	Bark 1, 2	1.3	1.35	0.0080	2.3	0.0050	2.0
	Bark trunk	9.6	10.02	0.1439	42.4	0.1043	42.8
	Root 1, 2	18.7	19.50	0.0489	14.4	0.0211	8.7
	Root trunk	33.7	35.20	0.1078	31.7	0.0717	29.5
Total		95.8	100.0	0.3399	100.0	0.2435	100.0
Tree average				0.35%		0.253%	
- PO <sub>4</sub>	Wood 1, 2	15.3	11.6	0.0673	5.8	0.0075	5.4
	Wood trunk	29.3	22.3	0.0439	3.8	0.0132	9.4
	Bark 1, 2	15.7	12.0	0.1962	17.0	0.0424	31.0
	Bark trunk	9.7	7.4	0.1057	9.1	0.0084	6.0
	Root 1, 2	24.5	18.5	0.4116	35.8	0.0294	22.0
	Root trunk	37.0	28.2	0.3256	28.5	0.0373	27.0
Total		131.5	100.0	1.1502	100.0	0.1382	100.0
Tree average				0.88%		0.105%	
- S	Wood 1, 2	14.8	7.4	0.0695	2.1	0.0203	3.4
	Wood trunk	47.4	23.7	0.1706	5.1	0.0388	6.5
	Bark 1, 2	16.2	8.1	0.4050	12.2	0.0533	9.0
	Bark trunk	19.8	9.9	0.4177	12.6	0.0796	13.4
	Root 1, 2	51.9	25.9	1.292	39.0	0.2486	41.9
	Root trunk	50.1	25.0	0.952	29.0	0.1518	25.8
Total		200.2	100.0	3.305	100.0	0.5926	100.0
Tree average				1.65%		0.296%	
Complete	Wood 1, 2	39.0	11.2	0.261	5.1	0.0581	5.7
	Wood trunk	81.8	23.5	0.253	5.0	0.1038	10.3
	Bark 1, 2	41.0	11.7	0.643	12.8	0.1845	18.4
	Bark trunk	31.3	9.0	0.422	8.3	0.0717	7.1
	Root 1, 2	83.1	23.8	2.227	44.4	0.3074	30.7
	Root trunk	72.0	20.8	1.224	24.4	0.2764	27.8
Total		348.2	100.0	5.030	100.0	1.0019	100.0
Tree average				1.44%		0.287%	
Original tissue	Top wood	23.5	44.0	0.176	32.5	0.016	18.0
	Top bark	5.31	10.0	0.095	17.5	0.018	20.0
	Root trunk	24.5	46.0	0.269	50.0	0.059	62.0
Total		53.3	100.0	0.540	100.0	0.093	100.0
Tree average				1.01%		0.175%	

TABLE X (Continued)

THROUGHOUT THE TREE (FRENCH PRUNE) AFTER TWO YEARS' TREATMENT

ASH		CaO		MgO		K <sub>2</sub> O		Fe <sub>2</sub> O <sub>3</sub>	
GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL
gm.	%	gm.	%	gm.	%	gm.	%	gm.	%
0.013	0.5	0.0013	0.3	0.00041	0.5	0.00187	0.3	0.00011	0.2
0.1965	7.7	0.0564	14.0	0.0069	8.6	0.0659	10.5	0.00168	3.5
0.0572	2.2	0.0149	3.7	0.0044	5.5	0.0123	1.9	0.0004	0.8
0.4800	18.8	0.1344	33.5	0.0196	24.5	0.1132	18.1	0.0062	13.4
0.8508	33.5	0.0342	8.5	0.0151	18.9	0.2174	34.7	0.0291	61.9
0.9436	37.3	0.1604	40.0	0.0337	42.0	0.2160	34.5	0.0094	20.2
2.5411	100.0	0.4016	100.0	0.0801	100.0	0.6263	100.0	0.0468	100.0
2.64%	.....	0.417%	.. . .	0.083%	.....	0.65%	... ..	0.048%	.....
0.2035	5.0	0.0376	4.0	0.0052	3.2	0.0345	4.3	0.0014	1.1
0.2051	5.1	0.0460	5.0	0.0049	3.0	0.0615	7.7	0.0017	1.7
0.8321	20.7	0.2512	27.2	0.0474	29.4	0.1570	19.8	0.0092	9.4
0.5626	14.0	0.2134	23.1	0.0253	15.6	0.1367	17.3	0.0042	4.3
1.0657	26.6	0.1190	12.9	0.0357	22.4	0.2031	25.6	0.0715	73.5
1.1479	28.6	0.2534	27.8	0.0422	26.4	0.1994	25.3	0.0099	10.0
4.0160	100.0	0.9203	100.0	0.1607	100.0	0.7922	100.0	0.0976	100.0
3.06%	.. .	0.70%	.. . .	0.122%	... ..	0.604%	.. . . .	0.074%	.....
0.1361	2.1	0.0263	2.1	0.0054	1.7	0.0235	2.1	0.0026	2.2
0.2464	3.9	0.0692	5.7	0.0111	3.5	0.0739	6.7	0.0033	2.8
0.761	12.1	0.2414	20.0	0.0333	10.7	0.1652	15.0	0.0047	4.0
1.138	10.1	0.4296	35.4	0.0711	22.9	0.1960	17.8	0.0047	4.0
2.673	42.4	0.2460	20.3	0.1162	37.3	0.3176	28.8	0.0882	75.2
1.352	21.4	0.1998	16.5	0.0741	23.9	0.3271	29.6	0.0140	11.8
6.307	100.0	1.2123	100.0	0.3112	100.0	1.1033	100.0	0.1175	100.0
3.15%	... ..	0.606%	.. . . .	0.155%	.. . . .	0.55%	... ..	0.058%	.....
0.312	2.9	0.0473	2.7	0.0113	2.6	0.0877	4.0	0.0034	2.6
0.449	4.1	0.1169	6.7	0.0163	3.6	0.1349	6.3	0.0055	4.3
2.009	18.6	0.5002	28.9	0.0820	18.4	0.4018	18.6	0.0098	7.7
2.003	18.5	0.5947	34.3	0.0838	18.5	0.3443	15.9	0.0078	6.2
4.155	38.6	0.2783	16.1	0.1479	31.8	0.7728	35.7	0.0872	68.6
1.872	17.3	0.1965	11.3	0.1044	22.1	0.4226	19.5	0.0134	10.6
10.800	100.0	1.7339	100.0	0.4457	100.0	2.1641	100.0	0.1271	100.0
3.10%	.....	0.49%	.... .	0.13%	.....	0.62%	.... .	0.036%	.....
0.188	18.6	0.035	11.3	0.0113	28.2	0.091	25.8	0.0003	2.0
0.337	33.2	0.104	33.3	0.0079	19.3	0.096	26.5	0.0029	20.4
0.490	48.2	0.171	55.2	0.0213	52.5	0.171	47.7	0.011	7.76
1.015	100.0	0.310	100.0	0.040	100.0	0.358	100.0	0.0142	100.0
1.91%	.....	0.58%	.....	0.075%	.....	0.675%	.....	0.0268%	.....

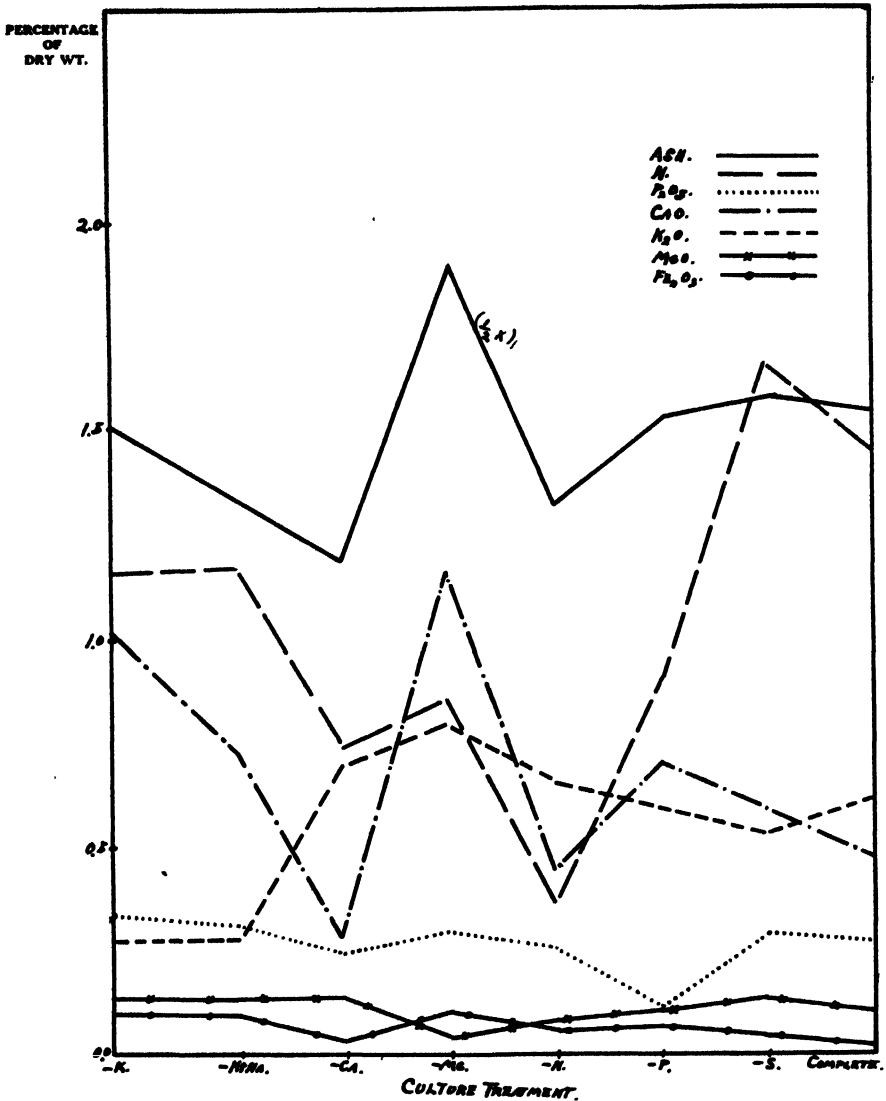


FIG. 3. Average mineral composition for the entire tree (in percentage of dry weight); French prune trees after two years of starvation treatment.

in the  $-K$  trees.  $MgO$  and  $K_2O$  contents were almost identical in the two groups.

Trees in the *minus-Ca* group had only one-half as high  $N$  (on per cent. dry weight) as had the complete-solution trees.  $P_2O_5$  was slightly low, ash was very low,  $CaO$  was 60 per cent. of the check (or normal),  $MgO$  was slightly above normal, and  $K_2O$  was about 16 per cent. above normal.

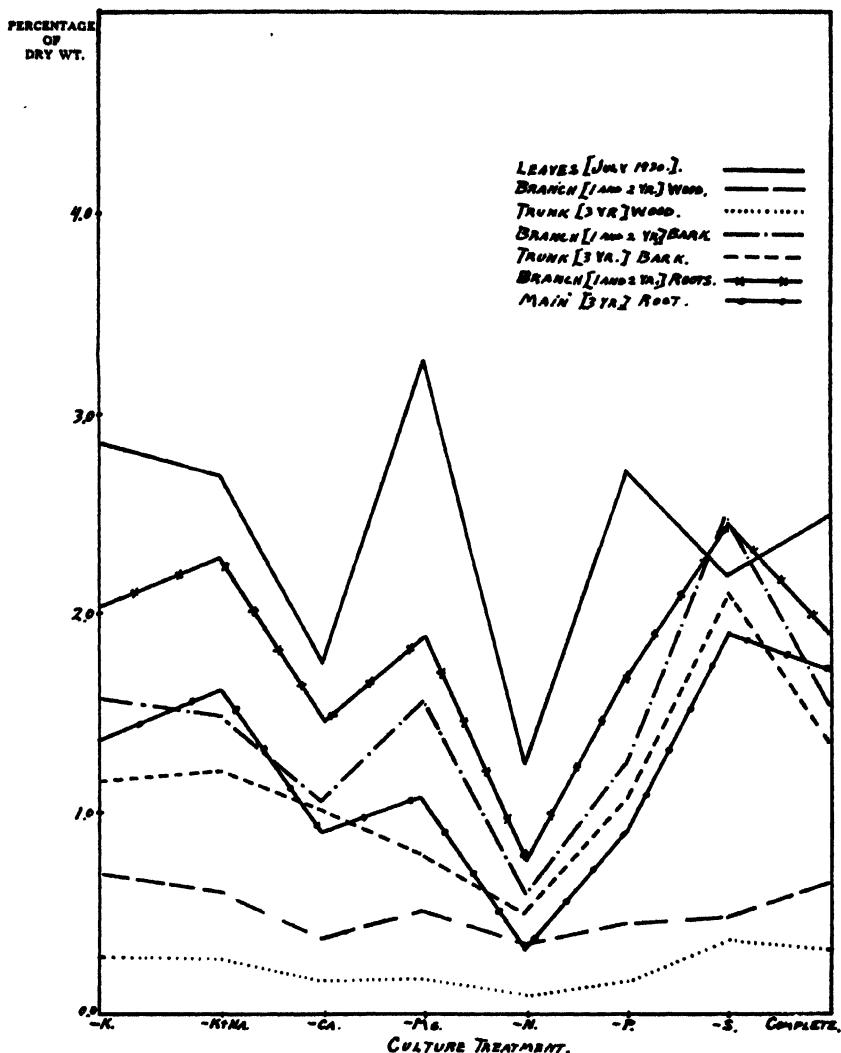


FIG. 4. Nitrogen content of various parts of French prune trees after two years of starvation for given elements.

Trees starved for *N* showed a content of *N* equal to 25 per cent. normal;  $P_2O_5$ ,  $CaO$ , and ash lower than normal;  $MgO$  far below and  $K_2O$  slightly above normal.

Trees starved for *Mg* had 59 per cent. of normal *N* content, very unevenly distributed throughout the tree, a normal  $P_2O_5$  content,  $CaO$  more than twice normal,  $MgO$  equal to 38 per cent. normal, and  $K_2O$  equal to 30 per cent. above normal.

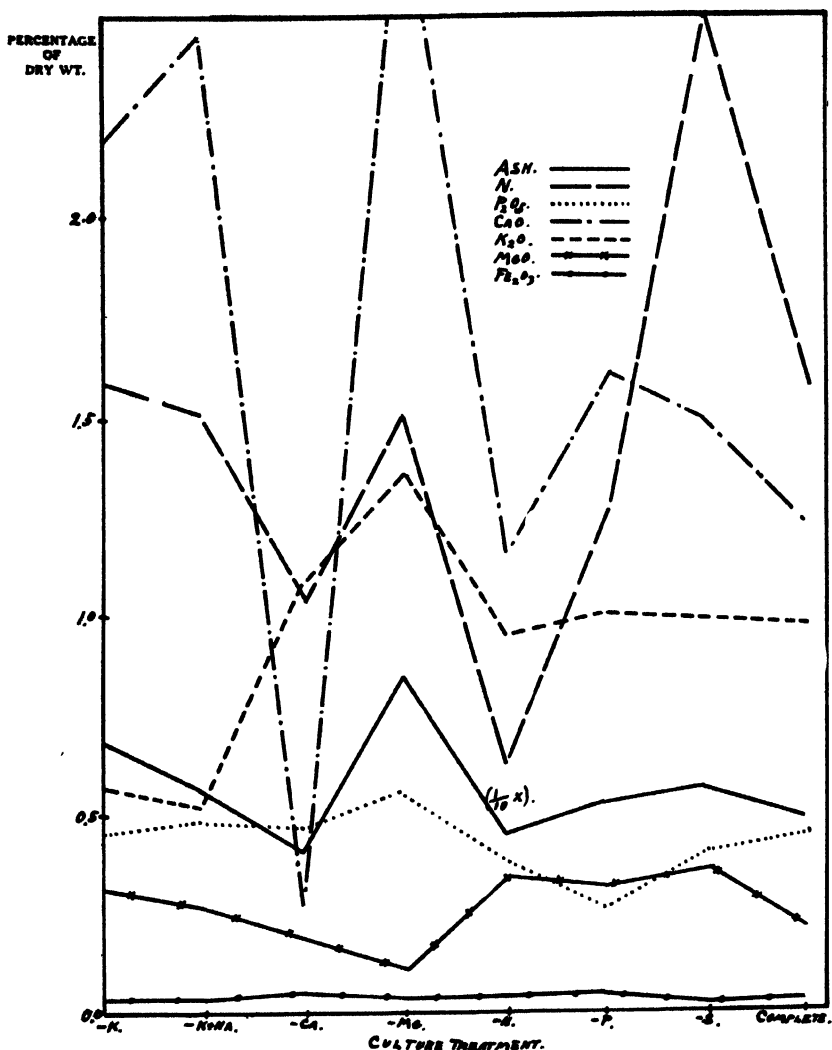


FIG. 5. Mineral composition in percentage of dry weight of branch (one and two year) bark tissues from French prune trees starved for given elements.

The N content of  $-P$  trees was only 61 per cent. normal,  $P_2O_5$  was 36 per cent. normal, ash was normal, CaO was 40 per cent. above normal, and MgO and  $K_2O$  were both somewhat lower than in the complete-solution trees.

*Minus-S* trees showed the highest N content of any trees in the experiment, being 14 per cent. above normal;  $P_2O_5$  was slightly above normal, as was also ash content, CaO, and MgO;  $K_2O$  was about 11 per cent. below normal.

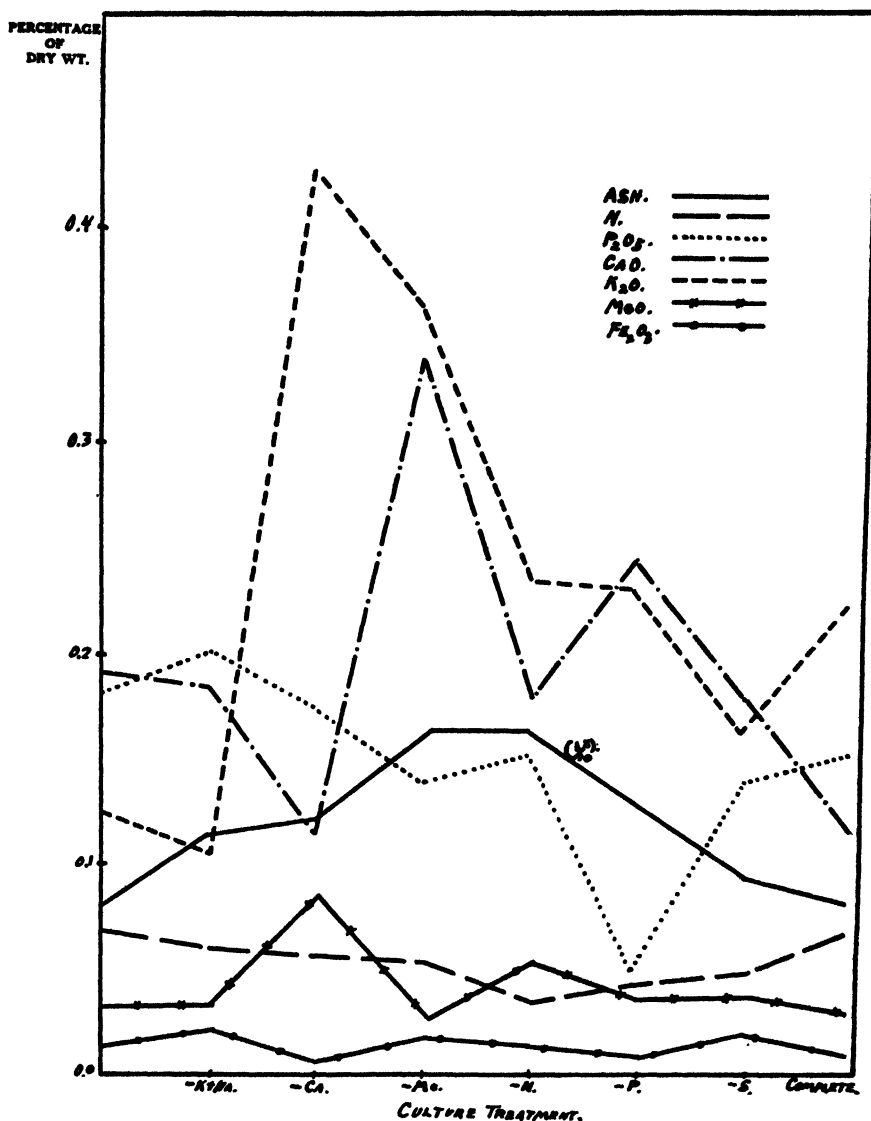


FIG. 6. Mineral composition in percentage of dry weight of branch (one and two year) wood from French prune trees starved for given elements.

Contrasting the analyses of the *complete* trees with those of original trees, we see that during the experiment these trees had gained in percentage N, P<sub>2</sub>O<sub>5</sub>, ash, MgO, and lost in CaO and K<sub>2</sub>O. It must be recalled, however, that the "original tree tissue" aliquot did not include the young tips of either top or root, as these young parts were pruned off before sampling

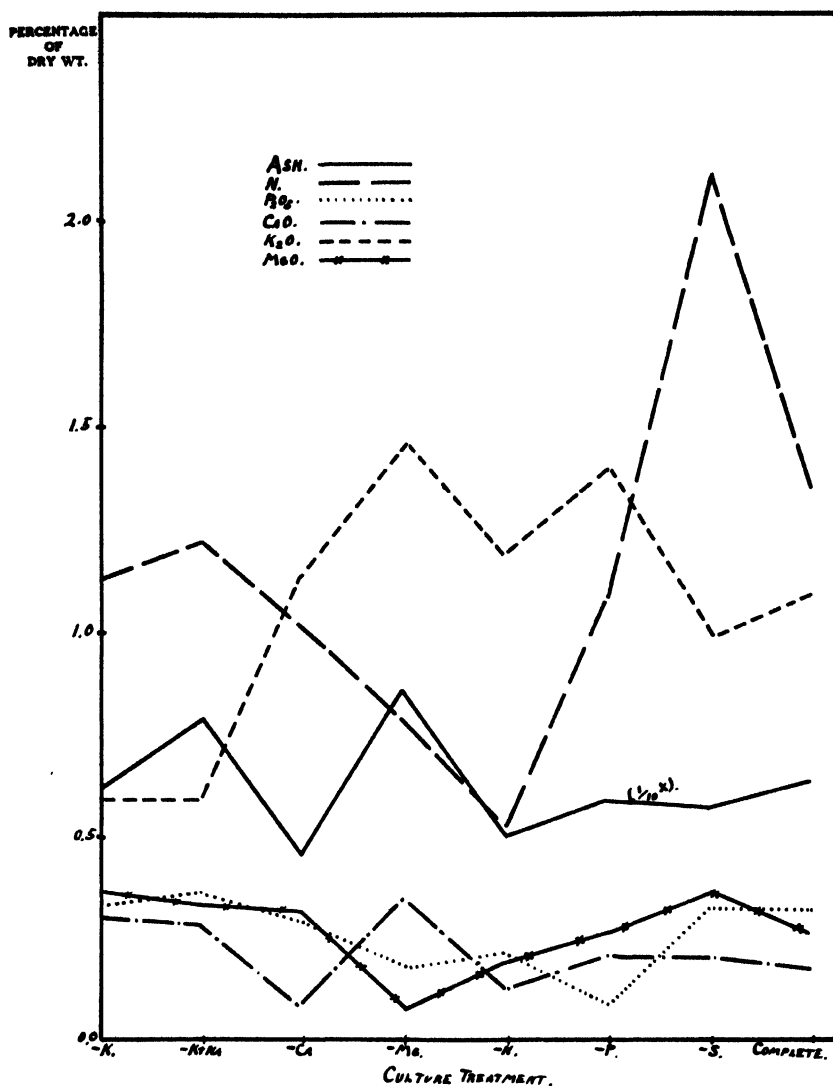


FIG. 7. Mineral composition in percentage of dry weight of trunk (three year) bark tissues from French trees starved for given elements.

was begun. Consequently the two sets of tissues are not entirely comparable. In the following section, parts of the trees (trunk and main root sections) that are entirely comparable, are discussed in detail.

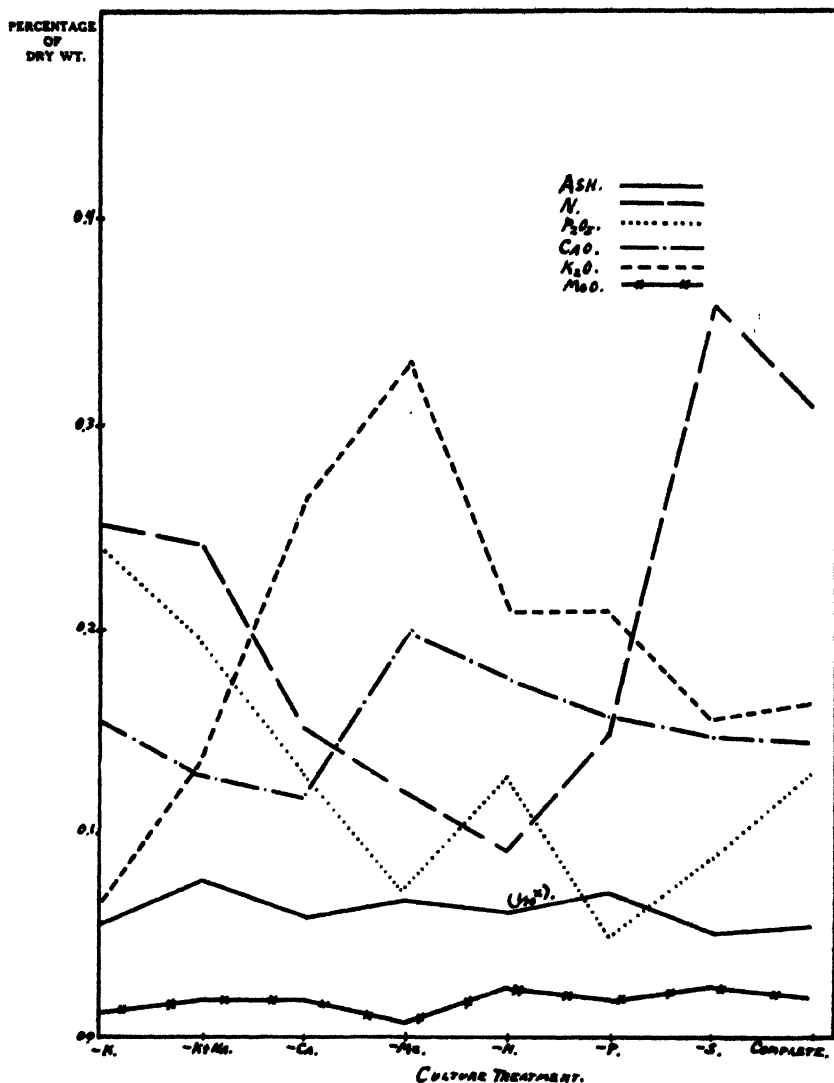


FIG. 8. Mineral composition in percentage of dry weight of trunk (three year) wood from French prune trees starved for given elements.

#### Distribution of mineral elements within the trees

As to the mineral composition of various parts of the trees of each group in the experiment, it appears (table X) that elemental starvation in these fruit trees caused considerable redistribution of mineral elements within the various organs of the tree.

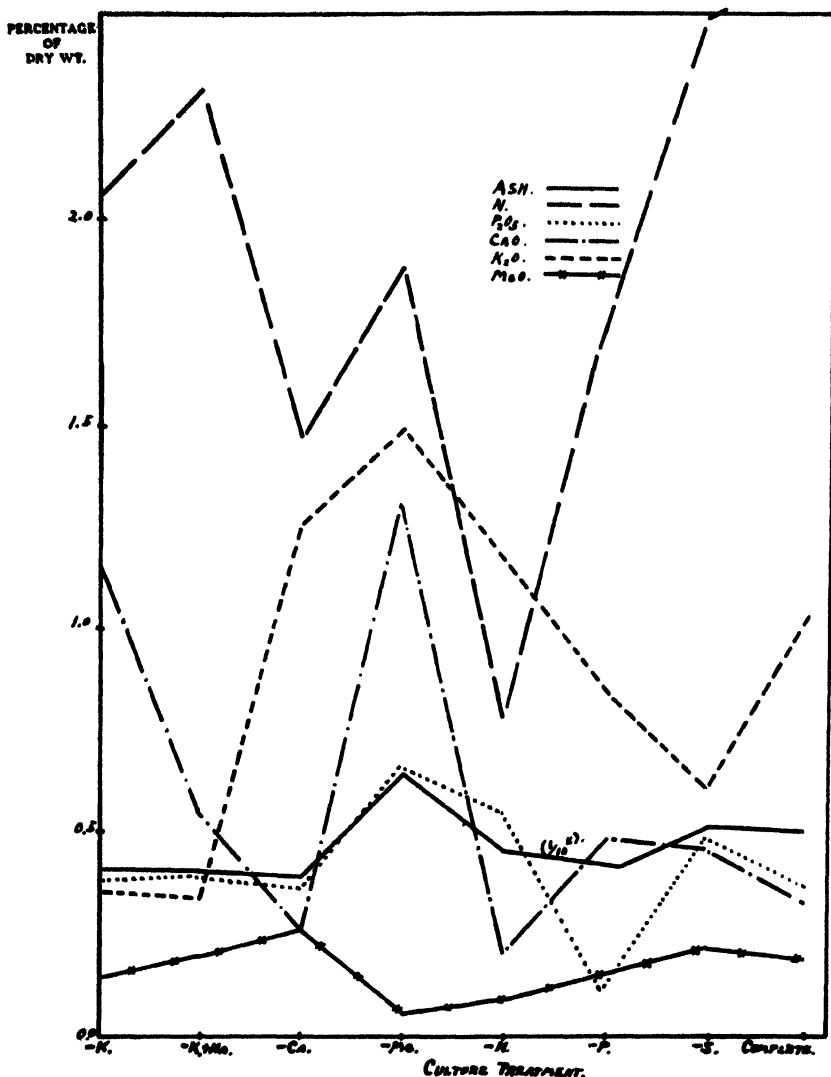


FIG. 9. Mineral composition in percentage of dry weight of branch (one and two year) roots from French prune trees starved for given elements.

*Minus-potassium group.*—The upper parts of these trees were high in N as compared with normal (or complete-solution) trees. The leaves (all leaf tissue referred to in this section was harvested July 25, 1930) especially were high in both N and P. The lower parts of the trees were low in N; the wood was high in P, and Fe<sub>2</sub>O<sub>3</sub> was two to three times more than normal. Young roots were high in calcium and low in Mg. The parts of the trees

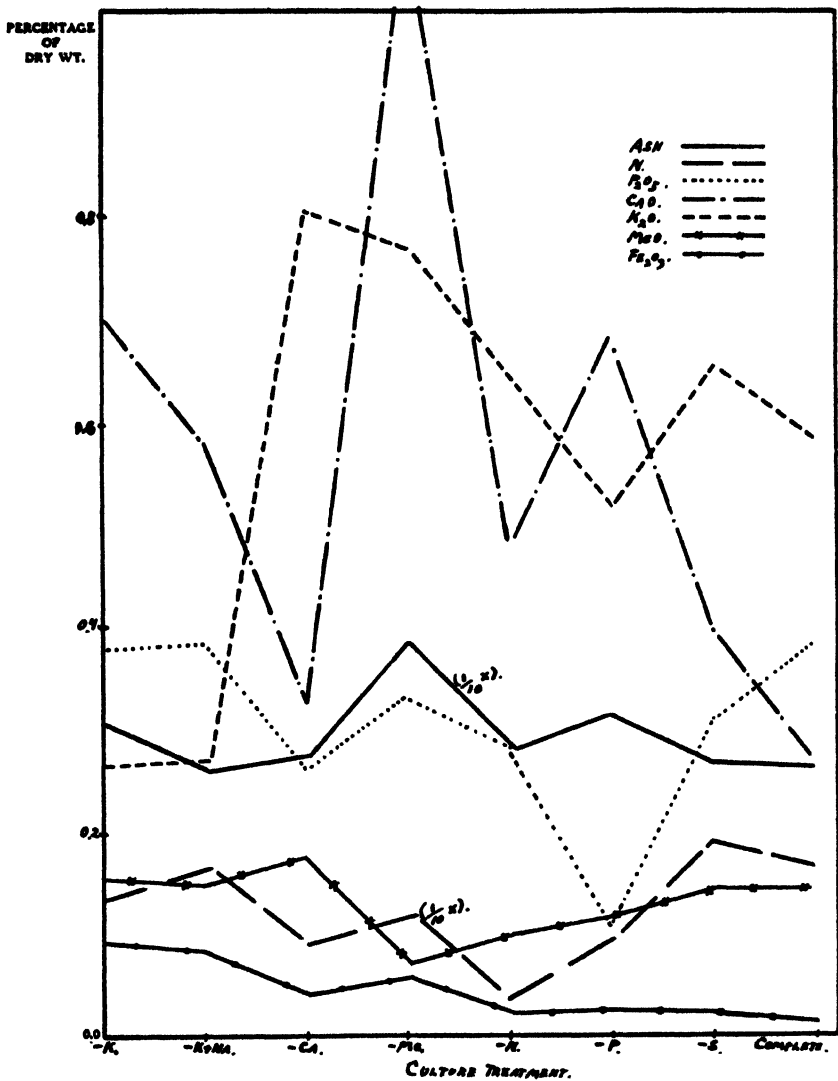


FIG. 10. Mineral composition in percentage of dry weight of main (three-year-old) root tissues of French prune trees starved for given elements.

lowest in K were: roots, about 41 per cent. normal in K; and trunk wood, 40 per cent. normal.

*Minus-potassium plus-sodium group.*—The composition was very much as in the preceding, but the entire were high in P. Perhaps this great absorption of P is characteristic of trees treated liberally with sodium salts.



FIG. 11. Distribution of dry weight among the various parts of two-year-old French prune trees at close of the starvation experiment.

*Minus-calcium group.*—The entire trees were low in N. Leaves showed only 35 per cent. of normal Ca content. Wood and main roots had abundant Ca, apparently immobile. Young bark showed a Ca content which was 21 per cent. of normal, apparently being the most severely Ca-starved tissue of the tree. Trunk wood was low in Fe.

*Minus-magnesium group.*—The leaves were high in N, the lower part of the tree being low in N; leaves were low in Ca, the rest of the tree being

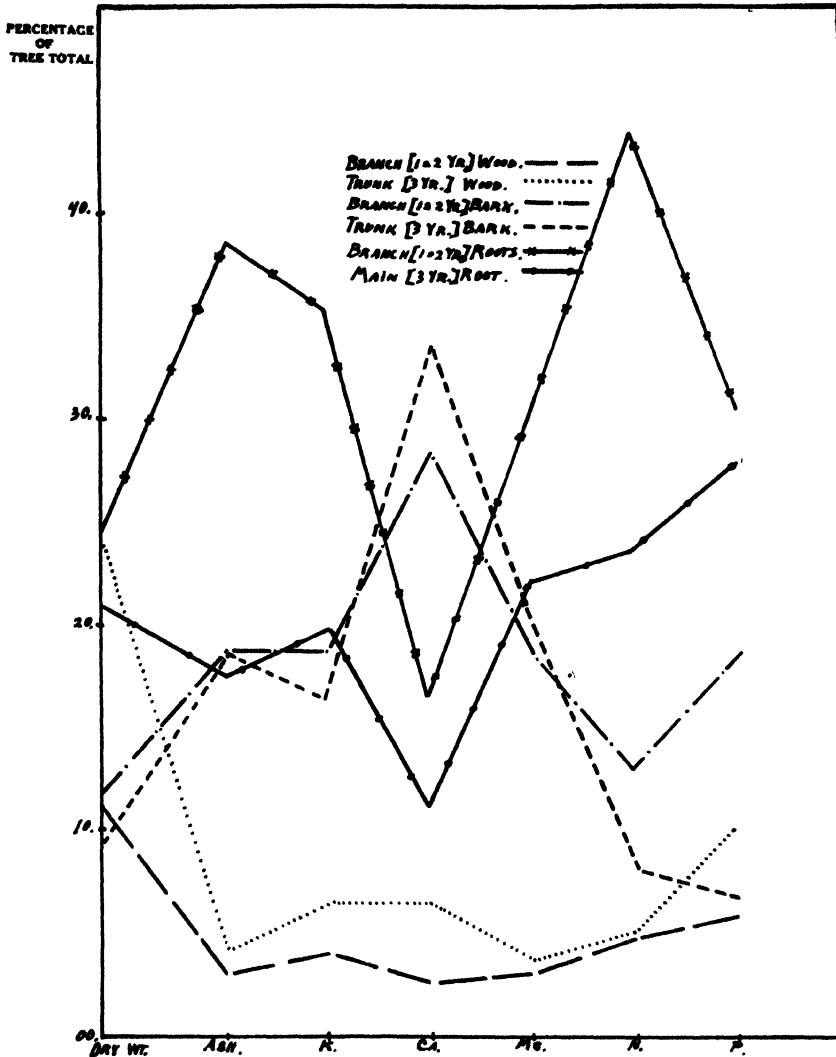


FIG. 12. Distribution of mineral elements among the various parts of French prune trees grown for two years in complete-culture solution; given in percentage of total weight of the given element in the tree.

high in that element. The leaves and young roots were high in P, but the rest of the tree was low in P. The wood was high in Fe. The entire tree was high in ash. The tissue lowest in Mg, in percentage of normal, was leaf tissue, with Mg 19 per cent. of normal.

*Minus-nitrogen group.*—The upper parts of the trees were high in Mg; the wood and young roots were high in K; wood and main roots were high

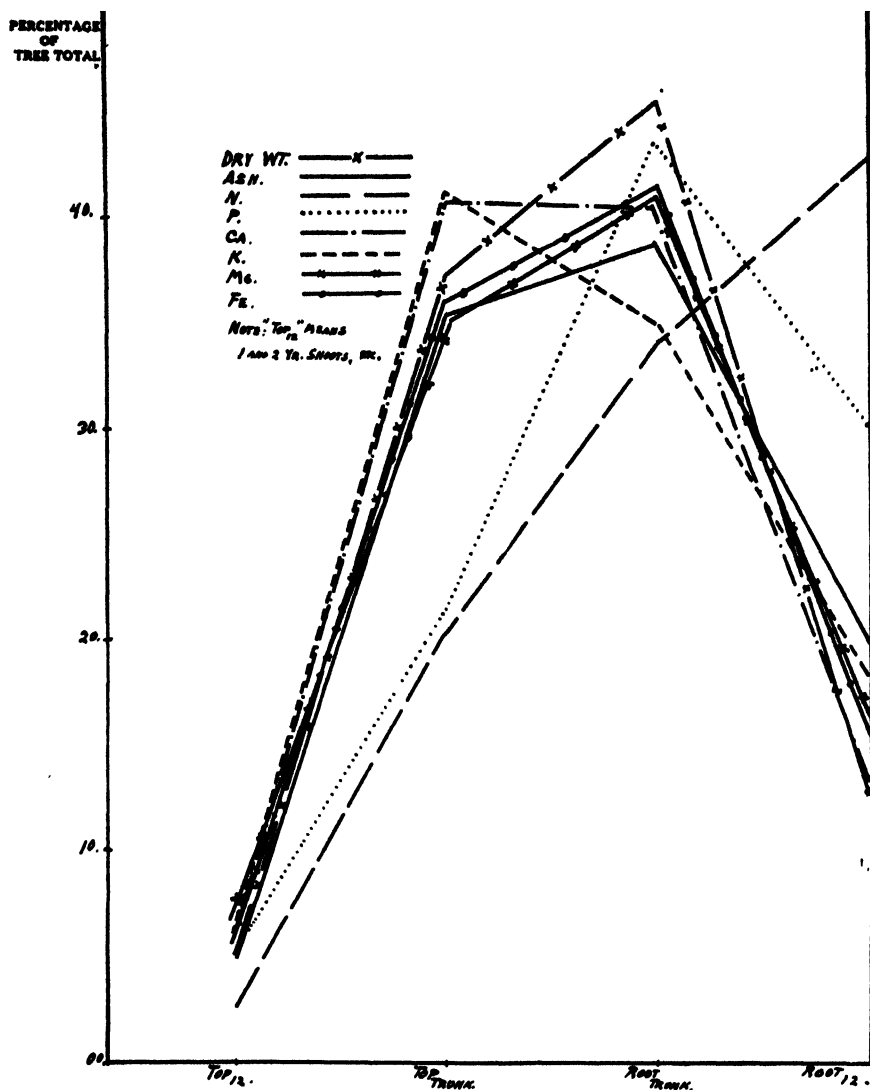


FIG. 13. Distribution of mineral elements among the various parts of French prune trees after two years of growth in distilled water.

in Ca; young roots and bark were low in Ca. The main root showed most severe N starvation, with a N content only 17 per cent. normal.

*Minus-phosphorus group.*—The leaves were high in N, but the remainder of the tree was low in this element. The upper parts of the tree showed high Mg, but the rest of the tree was low in it. The trunk parts were high

in Ca. The main root portion was severely starved for P, with only 26 per cent. normal content; trunk bark had 27 per cent. normal P, and leaf tissue 28 per cent.

*Minus-sulphur group.*—The young wood and leaves (July 25) were rather low in N, but the rest of the tree was very high in this element. The entire tree, except the young roots, was low in P. The upper parts of the trees were high in ash. Ca was high throughout the entire tree. Leaf tissue contained only about 15 per cent. of the normal S content.

## Distilled-water trees

French prune trees grown in distilled water for two years (tables IX-XIV) showed:

	PERCENTAGE OF NORMAL
Young branch (1 and 2 years) tops	$N = 27$ $\text{CaO} = 70$ $\text{K}_2\text{O} = 84$
Trunk top	$N = 39$ $\text{CaO} = 18$ $\text{K}_2\text{O} = 97$

Young roots showed N 55 per cent. of normal,  $K_2O$  55 per cent of normal, ash 76 per cent. of normal,  $MgO$  and  $CaO$  equal to that of complete-solution trees. Main roots had 19 per cent. normal N, 86 per cent. normal ash, low  $MgO$ , high  $CaO$ , and 50 per cent. normal  $P_2O_5$ .

It should be noted that the N content of the main root of these trees fell to the same level as in the main root of -N trees; but the young roots of the latter showed far more drastic N starvation than did young roots of distilled-water trees.

As to the complete-solution trees, it is to be noted that 45 per cent. of the dry weight of the tree was in the root at the close of the experiment (see fig. 11). With the exception of CaO, the bulk of the mineral total was in the root (leaves not being included). As for CaO, 63.2 per cent. of the tree total was in the bark of the top.

### Root-top distribution of mineral elements

**Roots of complete-solution trees contained:**

68.8	per	cent.	of	total	N	of	the	tree
58.5	"	"	"	"	P <sub>2</sub> O <sub>5</sub>	"	"	"
55.9	"	"	"	"	Ash	"	"	"
27.4	"	"	"	"	CaO	"	"	"
53.9	"	"	"	"	MgO	"	"	"
55.2	"	"	"	"	K <sub>2</sub> O	"	"	"

TABLE XI

## DISTILLED WATER TREES

TOTAL MINERAL CONTENT (GM. PER TREE) AND DISTRIBUTION AS SAME  
AVERAGE MINERAL CONTENT (ENTIRE TREE) AS PERCENTAGE OF DRY WEIGHT

DIVISION	GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL	GROSS	TOTAL
	DRY WEIGHT		ASH		N		Fe <sub>2</sub> O <sub>3</sub>	
	gm.	%	gm.	%	gm.	%	gm.	%
Top 1, 2 ..	4.0	4.2	0.124	4.9	0.0108	2.5	0.0012	6.3
Top trunk .	36.4	37.3	0.910	36.5	0.0873	20.5	0.0069	36.5
Root 1, 2 .	13.0	13.4	0.494	19.8	0.1820	42.8	0.0030	15.4
Root trunk ....	44.0	45.1	0.968	38.8	0.1450	34.1	0.0079	41.8
Total ..	97.0	100.0	2.496	100.0	0.4251	100.0	0.0190	100.0
Tree average .	.	.	2.57%	.	0.438%	..	0.0195	..

	CaO		MgO		K <sub>2</sub> O		P <sub>2</sub> O <sub>5</sub>	
Top 1, 2 . .	0.0190	5.6	0.0094	6.6	0.0199	5.5	0.010	5.1
Top trunk . .	0.1383	40.9	0.0508	35.7	0.1490	41.2	0.042	21.5
Root 1, 2 . .	0.0442	13.1	0.0231	16.2	0.0656	18.1	0.058	30.0
Root trunk . .	.1360	40.3	.0589	41.5	.1265	35.2	0.085	43.4
Total . . . .	0.3375	100.0	0.1422	100.0	0.3610	100.0	0.195	100.0
Tree average ...	0.348%	..	0.146%	.	0.372%	..	0.201%	.

Roots of distilled-water trees contained:

76.9	per cent. of	N	of the tree
73.4	" " "	P <sub>2</sub> O <sub>5</sub>	" " "
58.6	" " "	Ash	" " "
53.4	" " "	CaO	" " "
57.7	" " "	MgO	" " "
53.3	" " "	K <sub>2</sub> O	" " "

The iron content of the young wood (and of main roots) of -K, -K+Na, -Mg, and -S trees was excessively great, in proportion to the amount of Fe present in young bark tissues of these same trees. Iron appeared to have difficulty in reaching the bark under these conditions of starvation. On the other hand, young bark tissues of -P and -Ca trees were excessively high in iron, while the iron content of young wood and main root sections of these trees was comparatively very low. The work of INGALLS and SHIVE (4) on iron in lime-loving annual plants tends to confirm this point.

It should be stated here again that the -K, -K+Na, -Mg, and -S trees were all fed larger amounts of iron than were the complete, -Ca, or

– P trees. If all groups had been given equal amounts of iron, probably its total absorption by – K, – Mg, or – S trees would have been well below that by complete-solution trees.

All tissues analyzed for iron were washed in weak HCl at the time of harvesting the trees. In spite of this treatment, the small roots apparently retained large amounts of iron as impurity on the surface of the rootlets. The other tissues of the trees were washed very nearly free of iron contamination.

About May 15 of the second year of starvation, pH readings were made on freshly expressed sap from nearly mature leaves of the trees of each of the experimental groups. Sap was expressed almost immediately after the leaves were picked. The results are as follows (reading by colorimetric method):

		pH
Leaves from trees grown in	complete solution . . .	4.7
	– K “ . . . . .	5.3
	– K + Na “ . . . . .	5.0
	– Ca “ . . . . .	4.6
	– Mg “ . . . . .	5.2
	– N “ . . . . .	4.8
	– P “ . . . . .	5.1
	– S “ . . . . .	5.0

Elemental starvation was more effective in altering the pH of leaf sap than were changes in the pH of the culture solution itself. (Trees growing in solutions at pH 4 to pH 10 had leaves giving expressed sap of nearly identical pH.) At the same time (May 15) all leaves showed absence of nitrate ion, in appreciable quantity (by di-phenylamine test), except that low magnesium leaves, which though entirely green, showed at least 10–20 p.p.m. of nitrate in fresh tissue.

As to the general problems of fruit-tree nutrition and long continued growth of trees in water-culture medium, we still have much to learn before we can duplicate normal tree growth in fertile soil out-of-doors. Certainly aerated solutions are essential for fruit-tree culture work. LIVINGSTON has shown their desirability even for cultures of the comparatively smaller rooted annual plants. Also for maximum fruit-tree growth in water cultures an AZ type of solution will be necessary. HAAS (3) has recently reported that boron particularly is essential for normal growth of citrus seedlings. He described the symptoms produced on such trees by the absence of boron; for example, splitting and corking of the main veins of the leaves, together with other effects.

Much work has been done in the past in starving annual plants for one element at a time. A very interesting and little-known field lies open, in the study of starvation for two elements at a time, or the study of balance

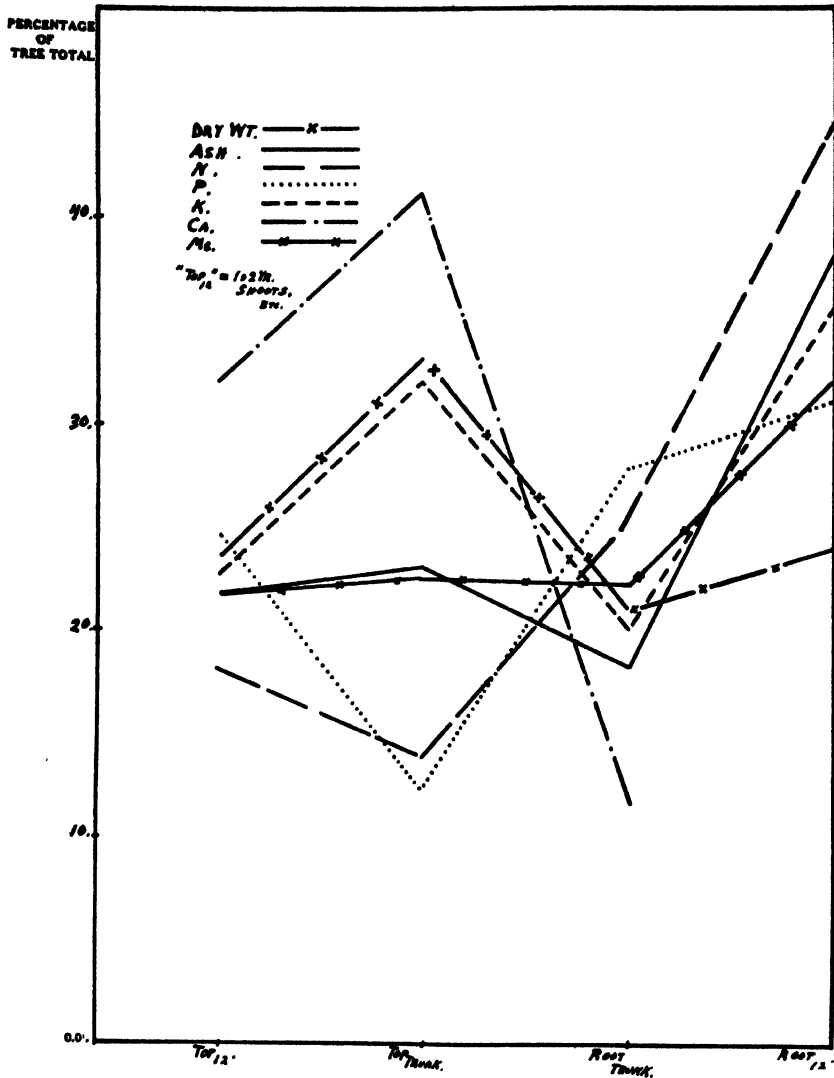


FIG. 14. Distribution of mineral elements among the various parts of French prune trees after two years of growth in complete solution (for comparison with figure 13).

between various ions. Already work has shown that a low-potassium plant also requires a low nitrogen supply in order to develop to full maturity and fruiting. In the writer's own experience, wheat plants low in both iron and sulphur developed to full maturity very quickly, heading out in normal fashion, but on short straw, even though the plants were always partially chlorotic. Plants low in both magnesium and iron offer interesting cases

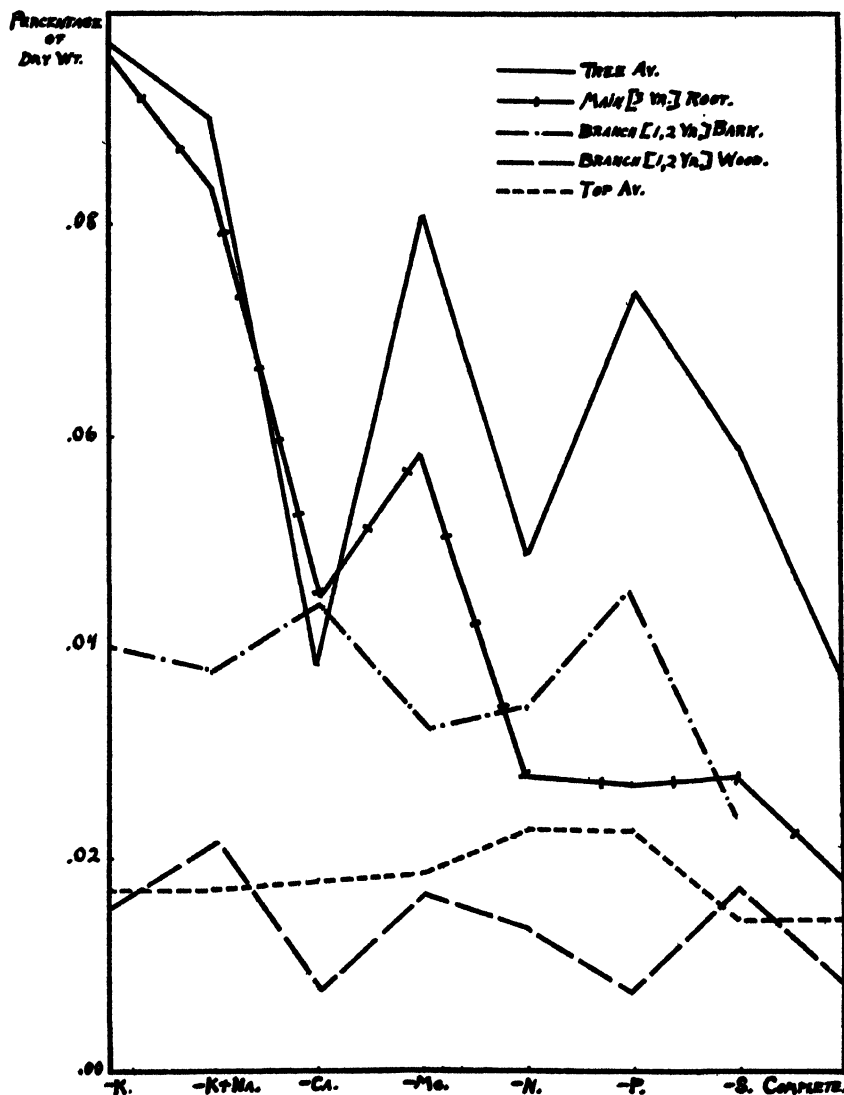


FIG. 15.  $\text{Fe}_2\text{O}_3$  content of various tissues of two-year-old French prune trees starved for given elements.

for study. The phosphorus-iron ratio in relation to the development of "bronzing" could well be studied.

It may be asked here, are we justified in studying such problems as the nutrition, elemental starvation, or seasonal absorption of fruit trees, as grown in water cultures in the greenhouse, as a means of throwing light on

the physiology of trees as they grow in the orchard? What factors appear to govern seasonal nutrient absorption by perennial plants? The salt absorption by tree roots early in summer is apparently governed by the amounts of storage, both organic and inorganic, in the tree; by the supply of soluble salts in the soil; by the pH of the soil solution, etc.; by temperature or light intensity; and by the rate of growth of both tops and roots. The very early growth of the roots (while the top is still dormant) probably explains the marked absorption of calcium early in the spring, when other elements are often actually lost from the roots, or at least are not absorbed to any extent.

Later in the season, when the storage supplies within the tree are depleted, the rate and character of absorption depend less on storage material but more directly on recent photosynthesis, shoot growth, leaf area, and therefore on light and temperature levels. Still later in summer, after terminal shoot growth is over and only diameter increase is proceeding, salt absorption is less rapid, and is perhaps not so greatly influenced by recent photosynthesis but more by stored food. It may be noted that much of the salt absorbed late in summer and in fall is calcium, with some sulphate (and in trees in soil, large amounts of silica and chlorides). These materials largely go to fill up the leaves, prior to their abscission, and are lost from the tree with the fall of the leaves. Consequently the excessive late-season absorption of calcium, sulphate, etc., has little importance in the metabolism of the woody part of the tree that goes into the winter.

Certainly the various species of fruit trees do not all make their shoot growth at exactly the same period of the summer, and salt absorption seems to vary in a similar manner. Young apple trees, especially in the coastal districts of California, start growth rather late in the spring, continue terminal shoot growth late into the fall, and hold part of their foliage until December, in some years at least. Such trees appear to show a far greater late-season absorption of nitrate than do the earlier maturing French prune trees, etc., in the same climate. BARKER (1) in England reports that most of the root growth of apple trees occurs after top growth is over.

### Summary

1. Starvation symptoms typical for each of the lacking elements were produced during the first year of starvation in French prune trees. Foliage symptoms were usually most strikingly characteristic and developed first, except in the case of calcium starvation, where root trouble is entirely dominant. Only with difficulty did such trees grow enough to show the leaf mottling characteristic of most low-calcium plants. Elemental starvation often produced changes in the mineral composition of leaf or bark tissues very different from those produced in wood, root, etc.

2. *Mg starvation* resulted not only in a low percentage of Mg in the leaves, but a Ca content actually lower than that found in - Ca leaves. Lack of Mg also resulted in high N and P in the leaves, very low N and P in the trunk wood, and very high ash content throughout the tree, except in leaf tissue.

3. *Ca starvation* resulted in both low N and low ash throughout the tree, except in the wood. Ca stored in the original trunk wood at the beginning of the experiment apparently remained there. The main root portion at the close of the experiment still showed abundant calcium, although leaf tissue had shown only 35 per cent. of normal calcium content.

4. *K starvation* resulted in leaf tissue high in N, P, Ca, Mg, and low in ash. Entire trunk parts and roots were low in N.

5. *N starvation* resulted in a low ash content except in the wood and very low N throughout. The young wood was peculiarly high in Ca and Mg, and normal in K and P. Young roots were low in Ca and Mg, and high in K. Main roots were extremely low in N (equal to 17 per cent. of normal), low in Mg and P, and high in Ca.

6. *P starvation* resulted in leaf tissue high in ash and N, extremely high in Mg and Fe, low in Ca, and  $P_2O_5$  equal to 28 per cent. of normal. All the other parts were decidedly low in N. The roots and trunk were low in Mg.

7. *S starvation* resulted in leaf tissue low in N and in ash, and a  $SO_3$  content equal to 15 per cent. of normal. The young wood was also low in N, but the rest of the tree was high in N, the average N content of the tree being 1.65 per cent. (dry weight basis), the highest of any group. Sulphur starvation decreased potassium absorption and increased absorption of Ca and Mg.

8. Trees grown in *distilled water* for two years had, at the conclusion of the experiment, a tree average of 30 per cent. normal N (on percentage of dry weight); 60 per cent. normal  $K_2O$ ; 71 per cent. normal  $CaO$ ; 70 per cent. normal  $P_2O_5$ ; and 54 per cent. normal  $Fe_2O_3$ . The final dry weight was 28 per cent. that of complete-solution trees of the same age.

9. As regards iron distribution in French prune trees grown in water culture, excessive amounts of iron occurred in young wood and in root portions of - K, - K + Na, - Mg, and - S trees. The iron content of the bark was excessive only in cases of P and Ca starvation, in which cases the iron in the young wood and main roots was proportionately very low.

10. It appeared possible that potassium starvation for one year might be more detrimental to subsequent growth and absorption than a like period of total starvation.

11. Trees placed in distilled water finally made far better growth of top and root than did like trees placed in a nutrient solution containing all the usual elements except calcium. Trees placed in a simple solution of calcium

hydrate at pH 7.2 made far better growth than did trees in a minus-calcium solution.

12. Trees grown for two years in minus-potassium solutions, and transferred April 12 of the third year to a plus-potassium solution, responded quickly and developed excellent shoot growth and fine leaf color in about two weeks. Only a trace of the former chlorosis remained near the midrib portions of the leaf blade. Leaf color was obviously improved five days after the potassium was added to the culture solution. Later in summer, however, the tip leaves of the young shoots of these trees showed excessive reddening, then browning or burning, and drying up. The shoot tip itself died back 6 to 8 inches.

13. Other trees, also previously starved for potassium for two years, and placed in complete solution cultures on May 15, or on June 25 of the third year, showed no shoot growth response whatever to the added potassium. Leaf color did change in about 10 to 12 days, however, and the non-scorched areas of badly chlorotic, scorched leaves turned green except for small areas bordering on the scorched margins of the leaf blade. The roots of these trees all responded vigorously to added potassium, quickly making abundant root growth. Apparently it is impossible after about May 1 to stimulate shoot growth in these trees by adding potassium.

14. The pH of the expressed sap of the leaves of the starvation series showed that elemental starvation is more effective in altering pH of leaf sap than are changes in the pH of the culture solution itself. (Trees growing in solution at pH 10 and pH 4 had leaves giving expressed sap of nearly identical pH.) Freshly expressed sap taken from nearly mature leaves of trees starved for K, S, or Mg showed higher pH readings than did sap from complete-solution leaves.

15. At the same time (May 15) all leaves showed absence of nitrate in appreciable quantity, except the low-magnesium leaves, which though entirely green, showed at least 10–20 p.p.m. of nitrate in fresh tissue.

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# DATE OF FLOWERING AS AFFECTED BY CLIMATIC TEMPERATURE

J. F. A. MACLAGAN

(WITH EIGHT FIGURES)

## Introduction

The investigation reported here is mainly concerned with the effect of temperature on the flowering date of a number of genera of woody shrubs. Since the reproductive phase of a plant is not independent of its vegetative phase, and since temperature does not act independently of other climatic factors, before considering the special aspect of the problem,—the time of onset of the reproductive phase as influenced by temperature,—it seems advisable to examine briefly the wider question of how the plant as a whole is affected by climatic conditions.

INFLUENCE OF CLIMATIC FACTORS.—The effect on the plant of climatic conditions is not clear-cut. One climatic factor rarely if ever exercises an effect independently of other external factors, climatic, soil, etc. The reaction of the plant itself is complex. Climatic factors operate by their influence on the metabolism of the plant, and the reactions of the various metabolic functions are not uniform. These interacting units of the environment and the plant's metabolism affect flowering both as to quantity and time.

The primary effects of these climatic variations express themselves in a broader sense in the relationship which exists between the vegetative and reproductive phases of the plant. In this broader sense any factor which tends to stimulate the development and therefore the continuance of the vegetative phase must retard the onset of the reproductive phase. The date of first flowering may be regarded as the first marked outward indication of the onset of the reproductive phase. If this be accepted, then from its variation from year to year, any correlation which can be established between weather and such variation may be taken as evidence of the effect of climatic factors on the relationship between the two phases. Actually the formation of the first definite flower initial marks the initiation of the change from vegetative to reproductive phase. This being so, date of flowering is in reality a late indication of this change of phase. No data are available on this fundamental point regarding the time when change of phase commences. Following initiation of change of phase are many other stages in the march of events toward actual flowering. Most significant of these steps is the initiation and formation of the actual gametes. It will be indicated later that it is at the moment of gamete formation that temperature exerts its maximum effect.

Previous work has been concerned mainly with amount of flowering and fruit setting in each year, rather than with date of flowering. While the quantitative side of flowering is not necessarily affected in the same way as time, nevertheless it is related.

BARKER and LEES (2) dealt with the effect of weather on fruit bud formation at Long Ashton. What is called good "ripening" or "maturing" of the wood occurs when vegetative growth ceases somewhat early in autumn, thus favoring the formation of flower buds (change of phase) before winter sets in. The comparatively wet July and August at Long Ashton caused active vegetative growth to continue late into the autumn so long as the temperature was high enough to favor it, and fruit bud formation suffered in consequence.

LEES (4) in a later paper says: "When the influence of pests, diseases, manuring, pruning and stock influence are eliminated, the remaining factors of previous crop and summer rainfall determine the future crop in the large majority of cases."

INFLUENCE OF TEMPERATURE.—It must be clearly realized that temperature, although dealt with here almost as an isolated factor for the purposes of this investigation, cannot strictly be separated from the other components of the environment. Similarly, time of flowering is influenced not only directly by the weather but also indirectly by the other processes taking place in the plant. Hence a perfect correlation of temperature with time of flower opening is not to be expected, although, as will be shown later, temperature is of major importance.

Previous workers have used the summation of all temperatures above an arbitrary datum line over a certain period to express the total amount of effective heat incident, and so to determine the dependence of plant development on this factor (for example, LIVINGSTON 5, and PALLADIN 6). The results arrived at by such a method are unsatisfactory. Temperature above a certain point may be non-effective or deleterious; the usually depressant or positively injurious effect of frost is ignored; and no allowance is made for the physiological effects of change in temperature at different temperature levels. Simple change of temperature within short periods has been shown to have a profound effect on plant development, at least at germination.

FLOWERING DATE AS INFLUENCED BY TEMPERATURE.—In the present investigation the weather records and flowering records have been compared with a view to finding some period or periods of the year when weather has a marked and consistent correlation with date of flower opening. Temperature has been found to exhibit such a correlation. The mechanism through which the effect of temperature at particular times operates has been sought

principally in the condition of the different parts of the flower bud, particularly the sporophylls, at these times. In this regard it is of interest to note that BALL (1), quoting GOFF as his authority, says in connection with the development of the flower buds of the different fruit trees: "The order of development is the same in the different fruits, *viz.*, first the calyx appears, then the petals, stamens, and finally the pistil. All these parts are present before the winter sets in, the ovules and pollen grains not being formed until February or March of the following year. There are exceptions to this since GOFF found that in the gooseberry ovules were generally present before the winter set in." BALL found that in the buds of three varieties of plum, Victoria, Monarch, and Pond's seedling, all the flower parts were present in November but ovules and pollen grains were not usually formed until January or February.

WHYTE (7) makes an illuminating observation in connection with the differentiation of floral parts. "The subject of time of reduction does not appear to have received much attention in the past, but from observations made by the writer on several unrelated genera of flowering plants, it may be said that in all those examined a considerable interval always occurs between the reduction processes in the pollen mother cells and the megaspore mother cells in any given flower. Growth of the female tissues does not generally commence until complete tetrads are formed, and pollen has been developed for some time before reduction in the ovules. The interval between the reduction processes is probably governed largely by the amount of ovular development in the plant concerned."

COULTER and CHAMBERLAIN (3) mention the time of formation of the microsporangia and megasporangia for species in which the details are known. In the case of the microsporangia, different species exhibit considerable variation. They suggest that for those plants whose flowers open early in the season the mother-cell stage is the usual winter condition. At any rate the interval of time between formation of the archesporium and pollination is probably in many cases considerably longer than is generally supposed.

In the winter bud of *Quercus velutina* the stamens are well formed but the tissue is still homogeneous. The microsporangia of *Salix glaucophylla*, *Populus monilifera*, and *Symplocarpus*, however, pass the winter in the mother-cell stage. A further advance is seen in *Alnus glutinosa* and *Corylus americana*, whose mid-winter catkins contain pollen ready for shedding, with the generative cell formed.

The length of time taken for the development of megasporangia is probably also very variable and related to the seasonal habit of the plant. CHAMBERLAIN has found that the megaspore mother cell of *Salix* and *Populus* is not formed until the winter dormancy of the plant is over

and growth renewed in spring. In *Erythronium* the mother-cell stage is reached by the end of November and persists until early spring. The mother-cell stage has been found in *Acer rubrum* in March, indicating that in this species also the mother cell is the condition in which the winter is passed. It is to be noted that in *Salix* and *Populus* the microsporangia pass the winter in the mother-cell stage, but the megaspore mother cell is not formed until spring, a considerable interval of time elapsing between the processes of formation in the two classes of spore, male and female.

### Records of data

The records of flowering dates on which this work is based are those kept at the Royal Botanic Garden, Edinburgh (unpublished). The records consist of lists of those plants which came into flower during each week of the year. Occasionally the entries have been made at fortnightly intervals instead of at the end of each week, and sometimes the period has been extended to three weeks, or even a month. Such extensions are most frequent in the case of early spring and late autumn records. The continuity of the records has been broken by the war and other causes, but sufficiently complete records are available for the years 1919 to 1929, excluding 1924. The temperature records used here are those taken at Edinburgh University and published in the monthly weather reports of the Meteorological Office, in conjunction with the daily weather records kept at the Royal Botanic Garden, Edinburgh (unpublished).

The data have been organized into graph form, and in each case the graph of the yearly flowering date has been plotted in juxtaposition to the graph of the significant weather belt or belts.<sup>1</sup> The coordinates of the temperature graph are: abscissae, years; and verticals, average temperature of significant belt. In the graphs of the date of flowering the abscissae are years, and the verticals are the deviation in each year from the average date of flowering over the period in question. The graphs are supplemented by tables giving in greater detail the data from which the graphs have been drawn.

Consideration of weather data more detailed than monthly averages has not been attempted except in a very few instances. Not only the action of other external factors, but also the relative lack of accuracy of the flowering records would tend to invalidate any attempt at a finer correlation if a more detailed analysis were carried out.

As might be expected from a general knowledge of the annual periodicity of plants, correlations between weather and flowering date appear

<sup>1</sup> "Weather belt" is defined as the weather obtaining over a period of time, from one to three months, which by analysis has been found to be significant in causing, by its deviation from normal, a deviation from the normal flowering time of each species.

most clearly in autumn and spring. As pointed out, many species form the flower buds in the autumn of the year previous to their opening. In such species "wood maturation" will have an important effect on the subsequent year's flowering. Rainfall is of importance as well as temperature, since drought causes the cessation of vegetative growth and thus allows the reproductive phase to commence.

As different stages of development are attained in the flower buds of different species before winter sets in, and the final stages are accomplished when activity recommences in spring, the autumn weather probably affects different species to different extents. An almost complete theoretical graded series can be erected in connection with degree of development of the reproductive phase in different species in winter.

At one end of the series are species which over-winter with the buds in the purely vegetative condition, no attempt at differentiation being made. Perennials of this nature must be only a single stage more complicated in their weather response than annuals. While the weather prior to winter may affect their physiological state and therefore presumably their subsequent flowering, it will be the weather of the spring (and summer) immediately prior to flowering which will be significant.

The next stage in the series is that of perennials which proceed to differentiation of buds prior to the onset of winter, but which cease differentiation at some stage before reduction and spore formation. Such plants over-winter with two classes of buds, leaf buds and incipient flower buds. The weather prior to over-wintering must affect such differentiation and therefore flower production in the following year. How far this is significant cannot be stated. Gardeners believe that a plant with formed flower buds can be, by appropriate manipulation of external factors in early spring, induced to change its "intentions," in so far that the flower buds do not produce flowers but return to vegetative growth. Such reversal might be possible in plants of the class falling into this grade, but it does not seem possible for plants of the next grade, which is that of perennials that over-winter with flower buds so differentiated that the sporophylls have assumed their definite character. A slightly more advanced stage is that when not only are the sporophylls definite in character, but the spores and gametes, at least on the microspore side, are definitely formed.

In perennials of the first class, autumn weather can have comparatively little effect on date of flowering in the following year. In those of succeeding classes, the weather of the autumn preceding any flowering year gains increasing importance as we ascend the series. In essence the statement may be made that, where the species proceeds in its development to a somewhat advanced stage of the reproductive phase before entering the winter dormant period, a significant weather belt must exist in the autumn

prior to the flowering year. Furthermore, as has already been indicated from the literature, and as will be shown in actual cases later, since the change of phase usually occurs in two steps (male development prior to over-wintering and development subsequent to over-wintering), two significant weather belts may exist. The weather belts coincide with the time of the respective steps in development. In the case of those plants which over-winter entirely in the vegetative phase, one major weather belt may be looked for in spring prior to flowering. Finally, the actual process of bud bursting and flower opening in all species must be conditioned to some extent by weather, and therefore another type of weather belt may be expected. The effect of frost on the opening buds is especially significant, more from the point of view of the records. The gardener observer, seeing frost-blasted buds, would probably not record the plant as having flowered, but would wait for a second crop of buds to open before entering the plant into the records. Three main significant weather belts may therefore be envisaged: (1) a belt occurring in autumn after one year's flowers have fallen, and far removed in time from the next flower opening, called here the *distal belt*; (2) another belt occurring in spring or early summer, nearer in time to date of flowering, called here the *proximal belt*; and (3) the weather just at or about flowering time, called here the *immediate belt*.

Soon after the work was commenced it was realized that, owing to the method of compiling the flowering records at intervals of a week or more, the experimental error in dealing with single species might be so large as to obscure the clear outlines. Consequently the genus was taken as the unit for comparison in order to establish the general rules governing its behavior if such existed. Only those species in each genus which were completely recorded were considered and incorporated. While it was felt that the members of a genus were related sufficiently closely to be thus dealt with as a unit, the possibility of single species showing individuality has not been lost sight of, and these are detailed when they occur.

While in general the results obtained point to temperature as a master factor in flower bud development, particular cases often cannot be explained on this basis. This is to be expected, however, in view of the fact that other factors come into play and may have a modifying, and sometimes an overriding, effect. For example, rainfall would affect the nitrate content of the soil and thus (or even by modified water supply) affect the inter-relationship between the vegetative and reproductive phases. Internal factors may also have a potent, although less easily defined, influence. For example, it is generally accepted that a year of profuse flowering is followed in the subsequent year by a paucity of flowers, an effect which seems to hold despite favorable external conditions in the second year. The ques-

tion of characters of a genetical nature cannot be considered here, and in any case the clear lines of their possible effect are blurred by the nature of the records. Further, the question of imposed rhythm might be pertinent, but the fact that the records of most species used are of long standing militates against this.

### Presentation of data

#### *RHODODENDRON*

It was realized at the commencement of the analysis that "maturation of the wood" in autumn is an important feature of this genus. A comparison of the autumn conditions, however, failed to show any correlation with time of flowering. On further study of the records it became clear that a weather belt occurs rather earlier in the year, about one to two months after flowering, which can be correlated with date of flowering in the following year. Unpublished data supplied by Miss F. B. MURRAY to the effect that the formation of pollen in the next year's flower buds may reach an advanced stage in development at a comparatively early date, within some two months after flower fall, strengthens this assumption, especially in view of the graded series already indicated. A striking case is seen in *Rhododendron souliei*, which in 1930 came into flower in the first week of June, and had flower buds with pollen formed by the first week of July.

As the species of *Rhododendron* considered here flower over a period of five months (February to June), the distal weather belt is not the same for all; hence the genus has been divided into three groups according to the time of flowering of the species. Each group consists of species which on the average flower practically together.

While the temperature at this distal weather belt showed a decided influence on time of flowering, it was found that in all species the correlation failed to hold in the years 1922 and 1923. This was accounted for when a proximal weather belt was taken into consideration. The average temperature of the subsequent January-March period was found to provide an explanation of these aberrations when it emerged as the proximal weather belt. Cytological evidence again supported the contention, for though the pollen may reach an advanced stage in the autumn, the development of the ovary does not take place until the spring. Indeed, reduction division may not take place until a very short time before flowering (unpublished data by Miss MURRAY).

Graphs are presented to illustrate this combined effect on time of flowering of the temperature at two different periods. In each group of species the most fully recorded species has been chosen to represent the group. In the graph of temperature for the distal belt, 1918 temperature has been

plotted on the same vertical as flowering for 1919, and so on, in order to facilitate direct comparison.

In addition to the graphs, tables are appended giving the complete data for all members of each group.

TABLE OF *RHODODENDRON* SPECIES

SPECIES	AVERAGE FLOWERING DATE
GROUP I:	
<i>R. barbatum</i> . . . . .	February 28
GROUP II:	
<i>R. metternichii</i> . . . . .	March 23
<i>R. ciliatum</i> . . . . .	March 28
GROUP III:	
<i>R. schlippenbachii</i> . . . . .	April 29
<i>R. vaseyi</i> . . . . .	May 4
<i>R. roylei</i> . . . . .	May 10
<i>R. decorum</i> . . . . .	May 11
<i>R. smirnowii</i> . . . . .	May 20

GROUP I: SPECIES FLOWERING IN FEBRUARY

Although only one species falls into this group, its agreement with the general behavior of the genus seems to justify its inclusion.

The distal temperature belt is April/May, the proximal temperature belt January/February. Figure 1 and table I refer to this group.

Comparing first the graphs of flowering time (fig. R1c) and April–May temperature of the preceding year (fig. R1a), it will be seen that, in general, high temperature corresponds to early and low temperature to late flowering, although this belt is separated from the actual flowering date by nearly nine months. In this species, however, as in most other species of the genus, in the years 1922 and 1923 the reverse was true, that is, flowering in 1922 was late and in 1923 early, while the temperature of the distal belt in 1921 was high and in 1922 low. Again in the year 1919 flowering was very late although temperature of the distal belt was average. Comparing the temperature graphs of the distal and proximal belts (fig. R1a and R1b), it will be seen that they agree except in those years of aberration 1919, 1922, and 1923. In other words, the proximal temperature had an overriding effect in these years. The extremely cold spring (proximal belt) of 1919 caused flowering to be late in that year, in spite of average warmth in the distal belt (April–May, 1918). The cold spring of 1922 had the same effect, and the comparatively warm spring (proximal belt) of 1923 made

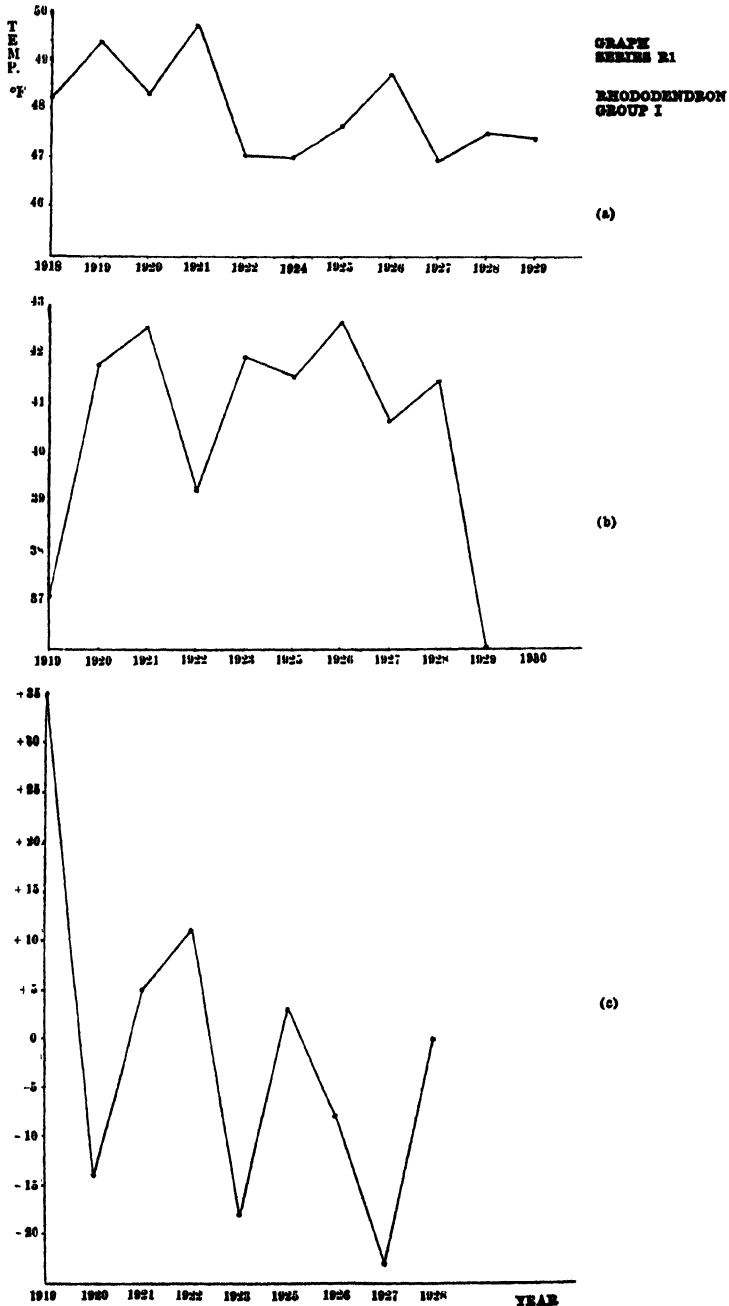


FIG. 1. Curves of distal temperatures (R1a), proximal temperatures (R1b), and deviations from average flowering time (R1c), of *Rhododendron barbatum*, 1918-1929.

flowering early in that year in spite of the less-than-average temperature of the previous April-May (distal belt).

TABLE I  
RHODODENDRON, GROUP I

YEAR OF FLOWERING	AVERAGE TEMPERATURE		<i>R. BARBATUM</i>	
	DISTAL (APRIL-MAY)	PROXIMAL (JANUARY-FEBRUARY)	DATE OF FLOWERING	DEVIATION FROM AVERAGE
1919	48.20	37.05	Apr. 5	+ 35.0
1920	49.35	41.75	Feb. 14	- 14.0
1921	48.30	42.50	Mar. 5	+ 5.0
1922	49.70	39.20	Mar. 11	+ 11.0
1923	47.05	41.90	Feb. 10	- 18.0
1925	47.00	41.50	Mar. 7*	+ 3.5
1926	47.65	42.60	Feb. 20	- 8.0
1927	48.70	40.60	Feb. 5	- 23.0
1928	46.95	41.40	Mar. 3	+ 0.5
1929	47.50	36.00		

\* Record made at end of a fortnight.

#### GROUP II: SPECIES FLOWERING IN MARCH

For this group the distal temperature belt is June, the proximal temperature belt January/March. Figure 2 and table II refer to this group.

For comparison of flowering date and weather, *R. metternichii* has been adopted as an example and its graph drawn. If the graphs of figure R2a (distal belt), R2b (proximal belt), and R2c (flowering curve) are compared, it will appear that here as in group I the correlation of flowering with distal temperature (in this case June) holds except in the years 1919, 1922, and 1923; and as in the case of group I, this is clearly accounted for by the temperature which obtained in the proximal belt affecting these years.

#### GROUP III: SPECIES FLOWERING IN APRIL AND MAY

The distal temperature belt affecting this group is June/July, and the proximal temperature belt is January/March. Figure 3 and table III refer to this group.

*R. vaseyi* has been chosen to exemplify this group, and graphed for purposes of comparison with the temperature belts. Figures R3a (distal tem-

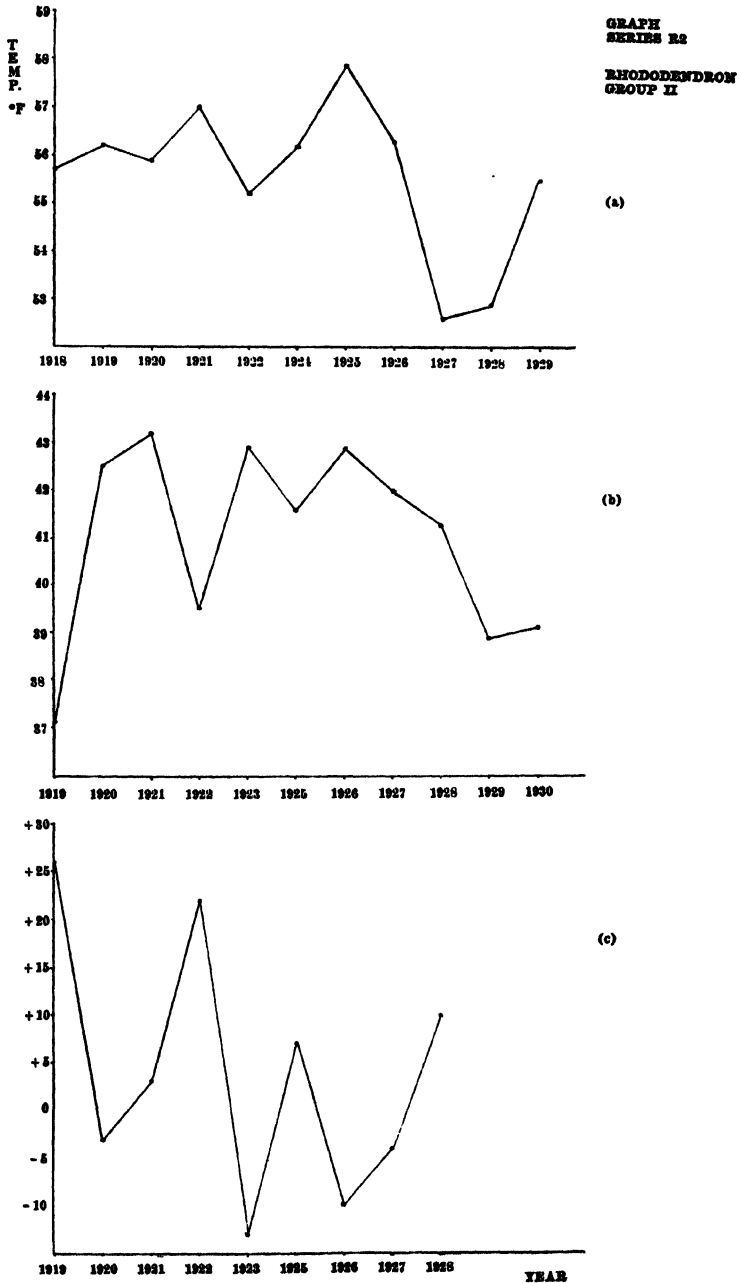


FIG. 2. Curves of distal temperatures (R2a), proximal temperatures (R2b), and deviation from average flowering time (R2c), of *Rhododendron metternichii*, 1918-1929.

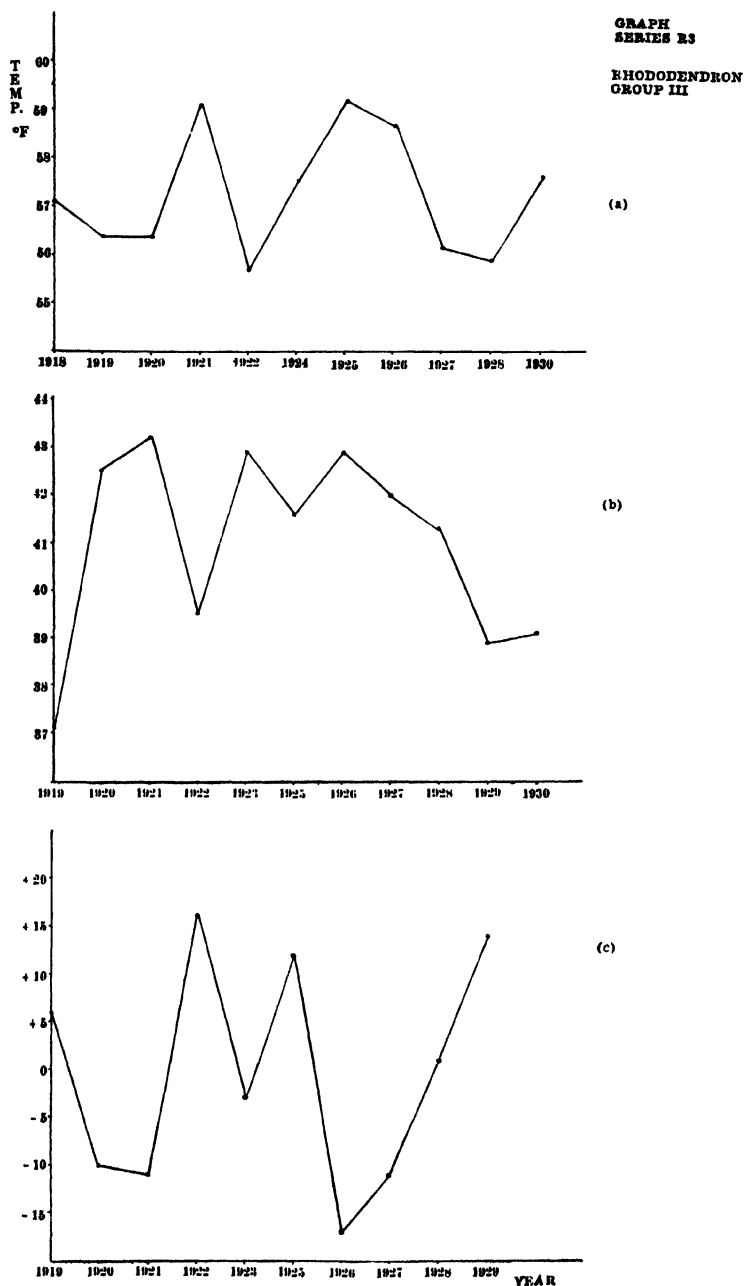


FIG. 3. Curves of distal temperatures (R3a), proximal temperatures (R3b), and deviation from average flowering time (R3c), of *Rhododendron vaseyi*, 1919-1929.

TABLE II  
RHODODENDRON, GROUP II

YEAR OF FLOWERING	AVERAGE TEMPERATURE		<i>R. METTERNICHII</i>		<i>R. CILIATUM</i>	
	DISTAL (JUNE)	PROXIMAL (JANUARY- MARCH)	DATE OF FLOWERING	DEVIATION FROM AVERAGE	DATE OF FLOWERING	DEVIATION FROM AVERAGE
1919	55.7	37.1	Apr. 19	+ 26.0	Apr. 19	+ 21.0
1920	56.2	42.5	Mar. 6	- 3.0	Mar. 20	- 8.0
1921	55.9	43.2	Mar. 19	+ 3.0	Mar. 26	- 2.0
1922	57.0	39.5	Apr. 8	+ 22.0	Apr. 15	+ 17.0
1923	55.2	42.9	Mar. 10	- 13.0	Mar. 10	- 18.0
1925	56.2	41.6	Apr. 4*	+ 7.5	May 2†	.. .. .
1926	57.9	42.9	Mar. 13	- 10.0	Mar. 13	- 15.0
1927	56.3	42.0	Mar. 19	- 4.0	Mar. 19	- 9.0
1928	52.6	41.3	Apr. 7*	+ 10.5	Apr. 7*	+ 5.5
1929	52.9	38.9	.	...	.. .	.. ..

\* Record made at end of a fortnight.

† Record made at end of three weeks.

perature), R3b (proximal temperature), and R3c (flowering date) show the same features as the graphs for groups I and II.

*CYTISUS*

This genus provides a contrast to *Rhododendron*, for in the case of *Cytisus* no distal temperature belt affecting time of flowering could be discovered. An examination of the flower buds of a number of species was made toward the end of February and no pollen was found. If, as seems likely, the flower bud passes the winter with the stamens and ovary in an undifferentiated condition, it is not to be expected that the autumn weather will have any marked effect on time of flowering. Thus in *Cytisus* no distal weather belt will occur.

TABLE OF <i>CYTISUS</i> SPECIES	
SPECIES	AVERAGE FLOWERING DATE

<i>C. kewensis</i>	April 20
<i>C. beanii</i>	April 21
<i>C. ardoinii</i>	April 21
<i>C. biflorus</i>	April 29
<i>C. purgans</i>	May 7

<i>C. decumbens</i> .....	May	8
<i>C. hirsutus</i> .....	May	12
<i>C. albus</i> .....	May	13
<i>C. horniflorus</i> .....	May	15
<i>C. versicolor</i> .....	May	17
<i>C. glabrescens</i> .....	May	21
<i>C. purpureus incarnatus</i> .....	May	22
<i>C. scoparius</i> .....	May	23
<i>C. scoparius prostratus</i> .....	May	23
<i>C. scoparius andreaeanus</i> .....	May	26
<i>C. purpureus albus</i> .....	May	28
<i>C. sessifolius</i> .....	May	29

#### CYTISUS, ALL SPECIES

The data show the month of March as the period during which temperature exercises its particular influence. Figure 4 illustrates this point. Figure 4, C1a, shows March temperature, and C1b is a composite graph for seventeen species of *Cytisus*. This composite graph is compounded from the average of the figures representing the deviations from the average flowering date shown in each year by each of the seventeen species. The figures for each individual species are given in table IV, columns 3 to 36; and the figures derived from these, from which the composite graph has been drawn, are shown in the last column of the same table. In finding the average for 1920, the figure for *C. horniflorus* has been ignored, since if it were included its enormous deviation ( $-70$ ) would give the average a false value. The two curves *a* and *b* show a remarkably close correlation, except that in the years 1923, 1927, and 1929 flowering was rather late. These deviations will be explained later by a more detailed consideration. Following these is a graph (fig. 4, C1c) for a single species, *Cytisus bearii*, which shows a remarkably perfect correlation with the temperature curve. As will be seen in table IV, the other species behave in the same way with minor deviations.

*Cytisus* as a whole seems to be rather an unstable genus, or perhaps easily affected by external conditions not evaluated here. There are a number of aberrations occurring in single species for which an explanation cannot be attempted. Such aberrations are very marked in the year 1929, and are the cause of lack of correlation between the composite graph (fig. 4, C1b) and the March temperature graph (fig. 4, C1a) in that year.

While the data do not permit explanation of these minor departures from the rule, two major departures may be accounted for on the general thesis developed here. These two deviations are seen, one in 1923 and the

TABLE III  
RHODODENDRON, GROUP III  
A, DATE OF FLOWERING; B, DEVIATION FROM AVERAGE DATE OF FLOWERING

YEAR OF FLOWERING	AVERAGE TEMPERATURE		R. SCHLIPPENBACHIII		R. VASEYI		R. ROYLEI		R. DECORUM		R. SMIRNOWII	
	DISTAL (JUNE-JULY)	PROXIMAL (JANUARY-MARCH)	A	B	A	B	A	B	A	B	A	B
1919 . . . . .	57.1	37.1	May 17	+18.0	May 10	+6.0	May 17	+7.0	May 17	+6.0	May 24	+4.0
1920 . . . . .	56.4	42.5	Apr. 24	-5.0	Apr. 24	-10.0	Apr. 17	-23.0	May 1	-10.0	May 8	-12.0
1921 . . . . .	56.4	43.2			Apr. 23	-11.0	May 7	-3.0	May 7	-3.0	May 7	-13.0
1922 . . . . .	59.1	39.5	May 13	+14.0	May 20	+16.0	May 20	+10.0	May 20	+9.0	May 27	+7.0
1923 . . . . .	55.7	42.9	May 5*	+2.5	May 5*	-2.5	May 5*	-8.5	June 2	+22.0	May 19	-1.0
1925 . . . . .	57.55	41.6			May 16	+12.0	May 25	+15.0	May 2†	-	May 25	+5.0
1926 . . . . .	59.2	42.9	Apr. 10	-19.0	Apr. 17	-17.0	Apr. 17	-23.0	May 1	-10.0	May 29	+9.0
1927 . . . . .	58.7	42.0	Apr. 16	-13.0	Apr. 23	-11.0	May 21	+11.0	Apr. 16	-25.0	May 7	-13.0
1928 . . . . .	56.16	41.3			May 5	+1.0	May 12	+2.0	May 19	+8.0	May 19	-1.0
1929 . . . . .	55.9	38.9			May 18	+14.0	May 18	+8.0	May 18	+7.0	June 1*	+8.5

\* Record made at end of a fortnight.

† Record made at end of three weeks.

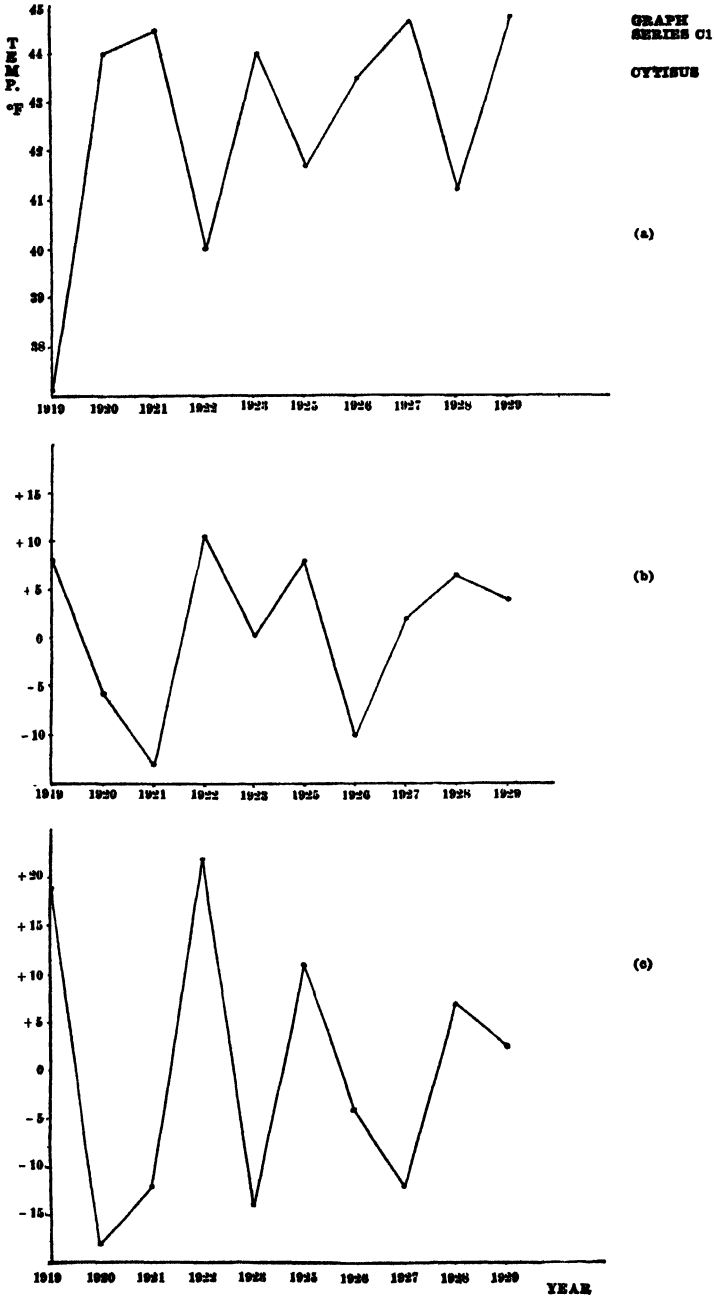


FIG. 4. Curves of March temperatures (C1a), composite deviation from average flowering time for 7 species of *Cytisus* (C1b), and deviation from average flowering time for *Cytisus beanii* (C1c), 1919-1929.

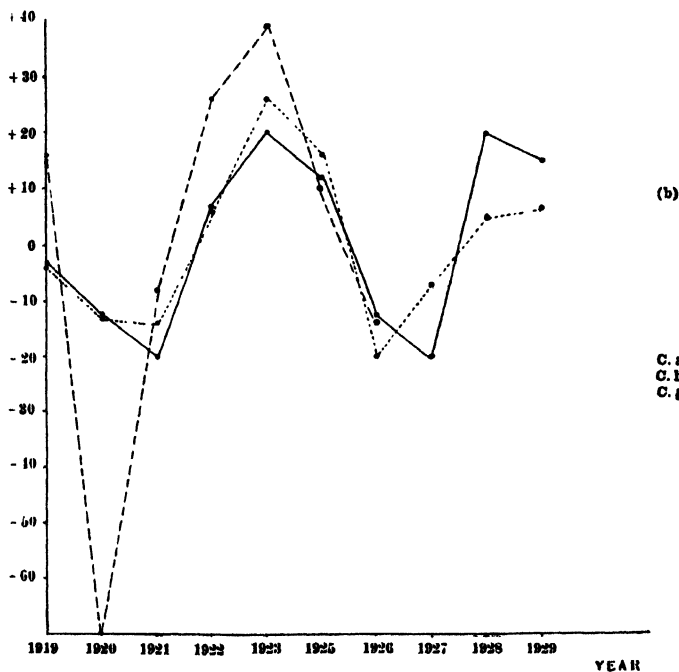
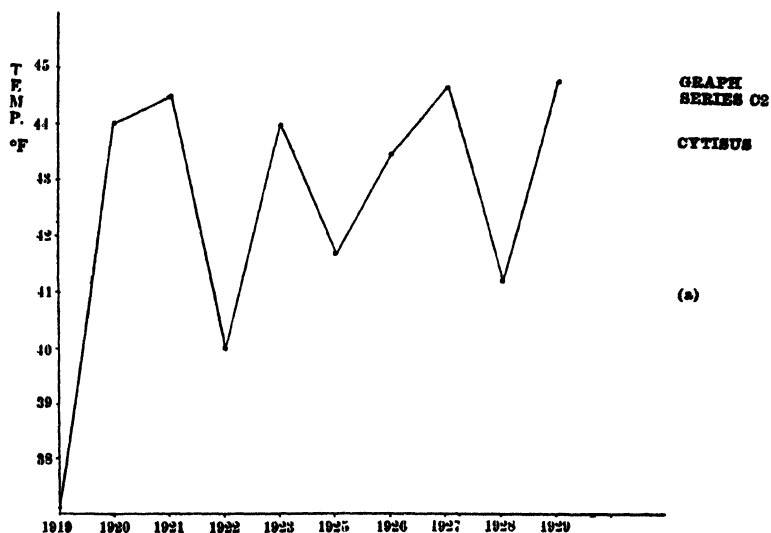


FIG. 5. Curves of March temperatures (C2a), and deviation from average flowering time for *Cytisus albus*, *C. horniflorus*, and *C. glabrescens* (C2b), 1919-1929.

other in 1927. In 1923 three species, *C. albus*, *C. horniflorus*, and *C. glabrescens*, differed from the others in flowering very late.

SPECIES	AVERAGE FLOWERING DATE	FLOWERING DATE IN 1923
<i>C. albus</i> . . . . .	May 13	June 2
<i>C. horniflorus</i> . . . . .	May 15	June 23
<i>C. glabrescens</i> . . . . .	May 21	June 16

This is illustrated in figure 5.

In 1927 other three species, *C. decumbens*, *C. versicolor*, and *C. scoparius*, flowered later than their fellows.

SPECIES	AVERAGE FLOWERING DATE	FLOWERING DATE IN 1927
<i>C. decumbens</i> . . . . .	May 8	May 28
<i>C. versicolor</i> . . . . .	May 17	May 28
<i>C. scoparius</i> . . . . .	May 23	June 4

This is illustrated in figure 6.

Those species which were retarded in 1923 are the reason for the lack of correlation exhibited by the composite graph of the genus in 1923, and similarly those species which were retarded in 1927 explain the lack of correlation of the composite graph in 1927. This is illustrated in table V, where alongside the composite figures for all seventeen species (column A) are seen two sets of composite figures for fourteen species (columns B and C), where in one case the three species which were late in 1923, and in the other case the three species which were late in 1927, have been omitted. It will be seen that these columns (B and C) show their greatest deviation from column A in 1923 and 1927 respectively. If the figure in column A is replaced in 1923 by the figure in column B, and in 1927 by the figure in column C, the disturbing influence of these retardations, caused, as will be seen later, by cold spells, will be eliminated, and the A figures will be seen to fluctuate in accordance with the March temperature in a much more consistent manner.

The retardations of these species are examples of the effect of cold weather just at time of flowering (immediate temperature belt). In 1923 a cold spell set in on May 9 and lasted until the end of the month. The average temperature for May in that year was 47.5° F., several degrees below the usual May temperature. In 1927, in the last week of April, there were severe ground frosts, sufficient to retard flowering. The grass minimum temperatures recorded at the Royal Botanic Garden, Edinburgh, for this period are:

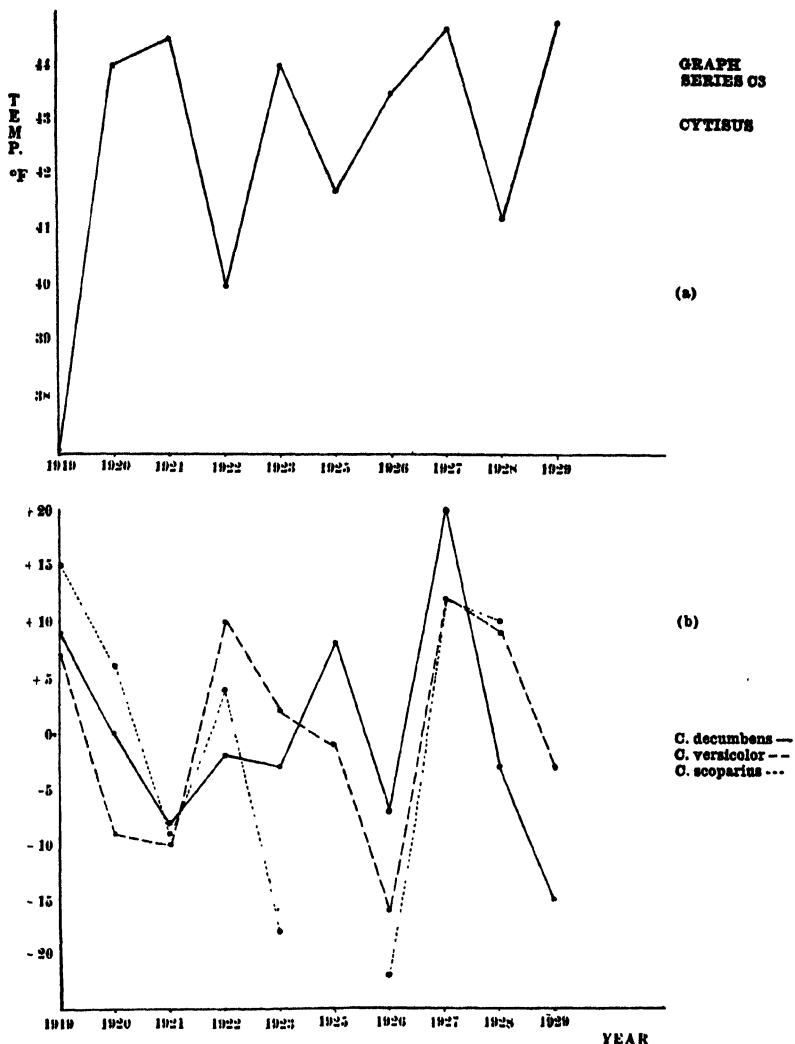


FIG. 6. Curves of March temperatures (C3a), and deviation from average blooming time for *Cytisus decumbens*, *C. versicolor*, and *C. scoparius* (C3b), 1919-1929.

	° F.
April 26 .....	23
April 27 .....	18
April 28 .....	14.5
April 29 .....	21
April 30 .....	24
May 1 .....	29

TABLE IV

*CYTISUS*  
A, DATE OF FLOWERING; B, DEVIATION FROM AVERAGE DATE OF FLOWERING

YEAR OF FLOWER- ING	AVERAGE TEMPERA- TURE (MARCH)	<i>C. KEWENSIS</i>		<i>C. BEANII</i>		<i>C. ARDOINII</i>		<i>C. BIFLORUS</i>		<i>C. PURGANS</i>		<i>C. DECUMBENS</i>		<i>C. HIRSUTUS</i>		<i>C. ALBUS</i>	
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1919	37.1	Apr. 26	+6	My. 10	+19	My. 3	+12	My. 10	+11	My. 17	+10	My. 17	+9	My. 22	...	My. 10	-3
1920	44.0	Apr. 10	-10	Apr. 3	-18	Apr. 17	-4	Apr. 24	-5	Apr. 24	-13	My. 8	0	My. 22	+10	My. 1	-12
1921	44.5	Mar. 26	-25	Apr. 9	-12	Apr. 2	-19	Apr. 9	-20	Apr. 30	-7	Apr. 30	-8	Apr. 23	-19	Apr. 23	-20
1922	40.0	Apr. 29	+9	My. 13	+22	My. 6	+15	My. 13	+14	My. 20	+13	My. 6	-2	Jn. 3	+22	My. 20	+7
1923	44.0	Apr. 7	-13	Apr. 7	-14	Apr. 7	-14	Apr. 14	-15	Apr. 21	-16	My. 5	-3	My. 5	-7	Jn. 2	+20
1925	41.7	My. 2	+12	My. 2	+11	My. 2	+11	My. 9	+10	My. 2	-5	My. 16	+8	Jn. 6	+25	My. 25	+12
1926	43.5	My. 1	+11	Apr. 17	-4	Apr. 17	-4	My. 1	+2	My. 1	-6	My. 1	-7	Apr. 24	-18	My. 1	-12
1927	44.7	Apr. 23	+3	Apr. 9	-12	Apr. 23	+2	Apr. 30	+1	My. 14	+7	My. 28	+20	My. 14	+2	Apr. 23	-20
1928	41.2	Apr. 21	+1	Apr. 28	+7	Apr. 28	+7	My. 5	+6	...	...	My. 5	-3	My. 5	-7	Jn. 2	+20
1929	44.8	Apr. 27*	+3.5	Apr. 27*	+2.5	Apr. 27*	+2.5	Jn. 1*	-1.5	Jn. 1*	+21.5	Apr. 27*	-14.5	Jn. 1*	+16.5	Jn. 1*	+15.5

<i>C. HORNIFLORUS</i>	A	B	<i>C. VESICOLOR</i>		<i>C. PURPUREUS INCARNATUS</i>		<i>C. GLABRESCENS</i>		<i>C. SCOPARIUS</i>		<i>C. SCOPARIUS PROSTRATUS</i>		<i>C. SCOPARIUS ANDREANUS</i>		<i>C. PURPUREUS ALBUS</i>		<i>C. SESSI-FOLIUS</i>		AVERAGE OF B COM- UMNS
			A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
My. 31	+16	My. 24	+7	My. 8	My. 17	-4	My. 8	-13	Jn. 7	+15	My. 24	+1	Jn. 14	+19	My. 31	+3	My. 31	+2	+8.25
Mar. 6	(-70)	My. 7	-9	My. 22	0	My. 7	-14	My. 29	+6	My. 8	-15	My. 22	-4	My. 22	-6	My. 29	0	0	-5.75
My. 7	-8	My. 7	-10	My. 14	-8	My. 7	-14	My. 14	-9	My. 28	+5	My. 7	-19	My. 14	-14	My. 14	-15	-15	-13.00
Jn. 10	+26	My. 27	+10	Jn. 3	+12	My. 27	+6	My. 27	+4	Jn. 3	+11	My. 27	+1	Jn. 3	+6	Jn. 3	+5	+5	+10.5
Jn. 23	+39	My. 19	+2	Jn. 2	-3	Jn. 16	+26	My. 5	-18	Jn. 2	+10	...	...	Jn. 2	+5	Jn. 2	+4	+4	+0.25
My. 25	+10	My. 16	-1	Jn. 6	+3	Jn. 6	+16	My. 25	+2	My. 25	+2	My. 25	-1	Jn. 6	+9	Jn. 6	+8	+8	+8.00
My. 1	-14	My. 1	-16	My. 28	-21	My. 1	-20	My. 1	-22	My. 1	-22	My. 28	+3	My. 22	-6	My. 15	-14	-14	-10.00
...	...	My. 28	+11	My. 28	+6	My. 14	-7	Jn. 4	+12	My. 28	+5	My. 28	+2	My. 28	0	My. 28	-1	-1	+2.00
...	...	My. 26	+9	Jn. 2	+4	My. 26	+5	Jn. 2	+10	My. 26	+1	Jn. 16*	+17.5	Jn. 2	+5	Jn. 16*	+14.5	+14.5	+6.50
...	...	My. 18*	-2.5	Jn. 1*	-7.5	Jn. 1*	+7.5	...	...	Jn. 1*	...	Jn. 1*	+2.5	Jn. 1*	+0.5	Jn. 1*	-0.5	-0.5	+4.00

\* Record made at end of a fortnight.

Considering these data as a whole, it is clear that in *Cytisus* there are two belts of weather (proximal and immediate) during which temperature affects date of flowering. The mechanism through which the temperature operates is not precisely known, and must await a cytological investigation. It may be that the two periods synchronize with spore differentiation; the earlier period with microspore development, and the later with megaspore production. This implies the existence of a time interval between the two processes which remains to be proved. On the face of the data, it seems likely that there is no definite cessation of activity at an intermediate point, but rather that the two processes are continuous, the one following the other without a definite interval; but that this extended period offers an extended time for temperature to operate, similar in aggregate length to that in the species showing two definite periods of spore differentiation activity, and that within this aggregate period the temperature may influence the two significant processes. The fact that the sharp lines of spore differentiation activity are in this way somewhat blurred accounts for the apparently fickle nature of the genus as a whole.

TABLE V

## CYTISUS

A, AVERAGE OF DEVIATIONS OF ALL 17 SPECIES FOR YEAR IN QUESTION; B, AVERAGE OF DEVIATIONS OF ALL SPECIES EXCEPT THOSE LATE IN 1923 (*C. ALBUS*, *C. HORNIFLORUS*, *C. GLABRESCENS*) FOR YEAR IN QUESTION; C, AVERAGE OF DEVIATIONS OF ALL SPECIES EXCEPT THOSE LATE IN 1927 (*C. DECUMBENS*, *C. VERSICOLOR*, *C. SCOPARIUS*) FOR YEAR IN QUESTION.

YEAR OF FLOWERING	AVERAGE TEMPERATURE (MARCH)	A	B	C
1919	37.1	+ 8.25	+ 9.50(1.25)*	+ 7.75(0.5)
1920	44.0	- 5.75	- 5.00(0.75)	- 7.00(1.25)
1921	44.5	-13.00	-12.75(0.25)	-14.00(1.0)
1922	40.0	+10.50	+10.00(0.5)	+12.00(1.5)
1923	44.0	0.00	- 6.25(6.25)	+ 1.50(1.5)
1925	41.7	+ 8.00	+ 7.00(1.0)	+ 8.75(0.75)
1926	43.5	-10.00	- 9.00(1.0)	- 9.00(1.0)
1927	44.7	+ 2.00	+ 4.00(2.0)	- 1.00(3.0)
1928	41.2	+ 6.50	+ 5.50(1.0)	+ 7.50(1.0)
1929	44.8	+ 4.00	+ 3.00(1.0)	+ 5.25(1.25)

\* Figures in brackets indicate difference between this value and the value for A.

## SYRINGA

When the table of deviations from average flowering date for this genus was drawn up, it showed a high degree of consistency for the genus as a whole. As in *Cytisus*, no distal temperature belt was discernible, neither

at first glance could a satisfactory proximal belt be found. The explanation was discovered in the marked overriding effect of the temperature at time of flowering (immediate temperature belt). When this belt was taken into consideration, *Syringa* was found to be governed, like *Cytisus*, by a proximal temperature belt in March, overridden by immediate temperature belts in April and May. Hence, although not at first evident, *Syringa* and *Cytisus* are very similar, although the immediate temperature belt has a more marked effect on *Syringa* than on *Cytisus*.

The twelve species of *Syringa* here considered fall into two classes according to their average time of flowering, hence in considering the genus two immediate temperature belts must be taken into account. The two species which have average flowering dates about the end of April/beginning of May (*S. villosa* var. *giraldii* and *S. pinnatifolia*) are governed by an immediate temperature belt in April. The other ten species, with average flowering dates at the end of May/beginning of June, are governed by an immediate temperature belt in May.

TABLE OF SYRINGA SPECIES

SPECIES	AVERAGE FLOWERING DATE
GROUP I:	
<i>S. villosa giraldii</i> . . . . .	April 22
<i>S. pinnatifolia</i> ..... ..	May 4
GROUP II:	
<i>S. vulgaris</i> . . . . .	May 24
<i>S. vulgaris alba</i> . . . . .	May 25
<i>S. persica</i> .. ... . .	May 25
<i>S. villosa lutece</i> .... .	June 1
<i>S. villosa</i> . . . . .	June 1
<i>S. reflexa</i> . . . . .	June 6
<i>S. yunnanensis</i> . . . . .	June 10
<i>S. adamiana</i> .. . . .	June 13
<i>S. emodii</i> .. . . .	June 17
<i>S. emodii variegata</i> ..... ..	June 25

#### GROUP I: SPECIES FLOWERING IN LATE APRIL AND EARLY MAY

For these species March is the proximal temperature belt, and April the immediate temperature belt. Figure 7 and table VI refer to this group.

Examining figure 7, it will be seen that the curves of *S. villosa* var. *giraldii* and *S. pinnatifolia* (S1c), excluding *S. pinnatifolia* in 1923, follow the March temperature curve S1a rather consistently, the most significant deviations occurring in 1927 and 1929. Time of flowering in 1927, and

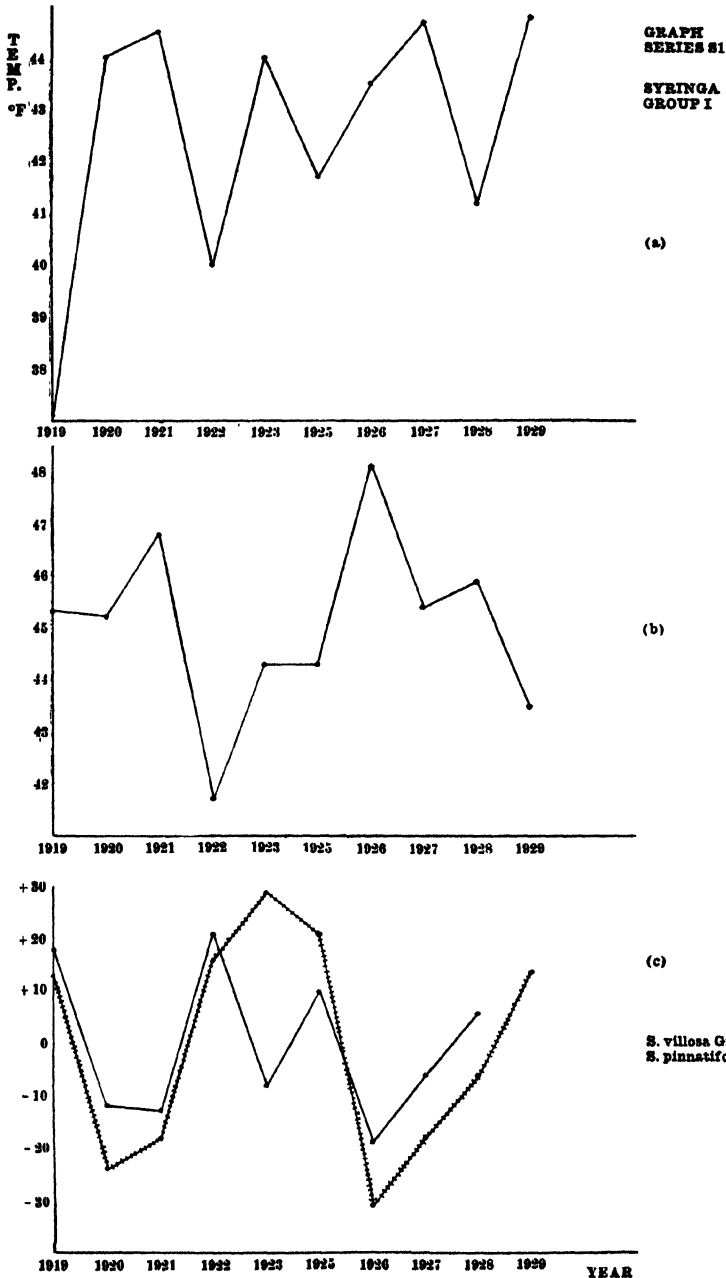


FIG. 7. Curves of March temperatures (S1a), April temperatures (S1b), and the deviation from average flowering time of *Syringa villosa giraldii* and *S. pinnatifolia* (S1c), 1919-1929.

more especially in 1929, was later than might be expected from the high temperature during March in those years. This is accounted for by the overriding effect of the April temperature (immediate belt), which in 1927 was average and in 1929 low. The only other years in which the April temperature curve (S1b) deviates from the March temperature curve (S1a) are 1919 and 1923. Comparison with the curve of flowering time (S1c) shows that in the former year April temperature has slightly modified the effect of March temperature. A point of more interest is the effect of the temperature in April, 1923, which was slightly below average. Apparently it had no effect on *S. villosa* var. *giraldii*, and a disproportionately powerful effect on *S. pinnatifolia*. A more detailed study of the weather conditions during April and May supplies the explanation. The temperature during the early part of April, although somewhat low, was not sufficiently low to retard the earlier flowering species *S. villosa* var. *giraldii*, which in consequence of a warm March flowered by April 14. Subsequent sharp frosts on April 15, 18, 27, and 28 checked *S. pinnatifolia* and held up its flowering so that it came under the influence of an extremely cold period which set in on May 9. Thus *S. pinnatifolia* in 1923 was four weeks late in flowering, and in this year should really rank as one of the later-flowering group with an immediate temperature belt in May.

TABLE VI  
SYRINGA, GROUP I

YEAR OF FLOWERING	AVERAGE TEMPERATURE		<i>S. VILLOSA GIRALDII</i>		<i>S. PINNATIFOLIA</i>	
	MARCH	APRIL	DATE OF FLOWERING	DEVIATION FROM AVERAGE	DATE OF FLOWERING	DEVIATION FROM AVERAGE
1919	37.1	45.3	May 10	+18	May 17	+13
1920	44.0	45.2	Apr. 10	-12	Apr. 10	-24
1921 . . .	44.5	46.8	Apr. 9	-13	Apr. 16	-18
1922 . . . .	40.0	41.7	May 13	+21	May 20	+16
1923	44.0	44.3	Apr. 14	-8	June 2	+29
1925	41.7	44.3	May 2	+10	May 25	+21
1926 ..	43.5	48.1	Apr. 3	-19	Apr. 3	-31
1927	44.7	45.4	Apr. 16	-6	Apr. 16	-18
1928	41.2	45.9	Apr. 28	+6	Apr. 28	-6
1929 ..	44.8	43.5	. . .	. . .	May 18	+14

GROUP II: SPECIES FLOWERING IN LATE MAY AND JUNE

Here the proximal temperature belt is March and the immediate temperature belt May. Figure 8 and table VII refer to this group.

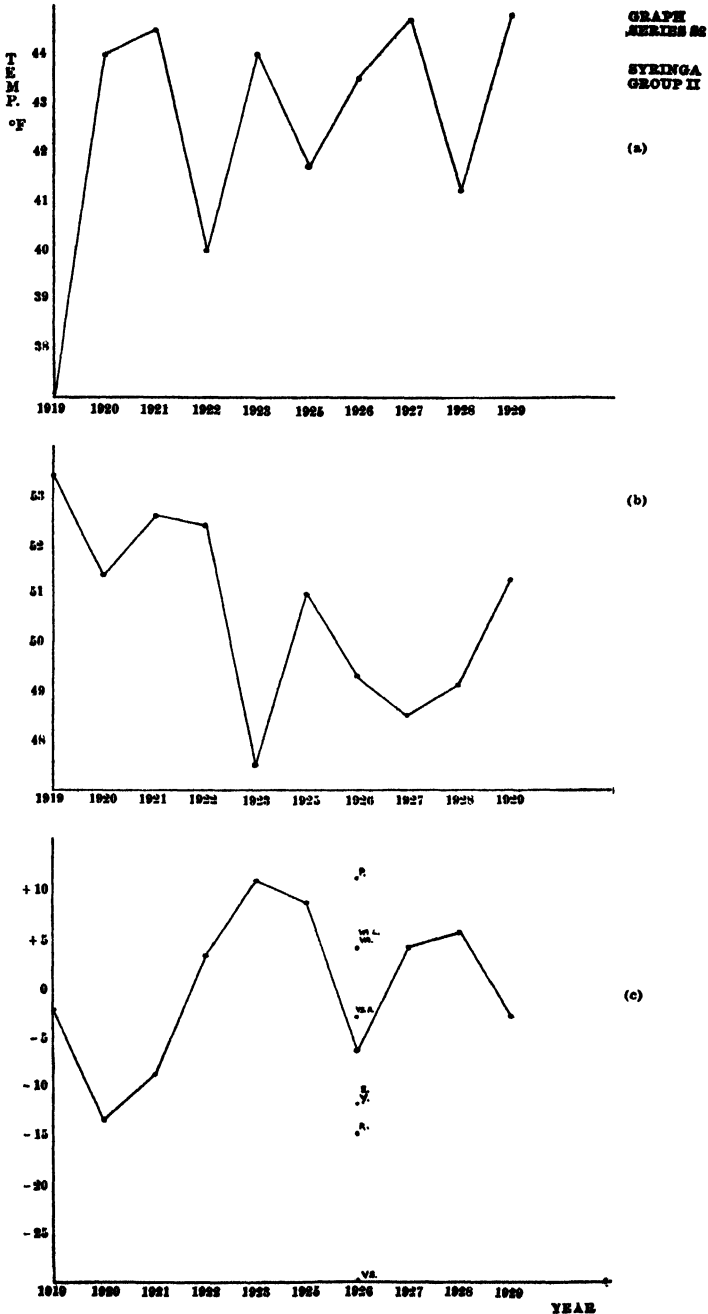


FIG. 8. Curves of March temperatures (S2a), May temperatures (S2b), and composite deviation from average blooming time for 10 species of *Syringa* (S2c), 1919-1929.

TABLE VII

SYRINGA, GROUP II

A, DATE OF FLOWERING; B, DEVIATION FROM AVERAGE DATE OF FLOWERING

YEAR OF FLOWERING	AVERAGE TEMPERATURE		<i>S. VULGARIS</i>		<i>S. VULGARIS ALBA</i>		<i>S. PERSICA</i>		<i>S. VILLOSA LUTESCENS</i>		<i>S. VILLOSA</i>	
	MARCH	MAY	A	B	A	B	A	B	A	B	A	B
1919	37.1	53.4	May 24	0	May 24	-1	May 24	-1	May 15	-17	May 31	-1
1920	44.0	51.4	May 22	-2	May 8	-17	May 15	-10	May 4	+3	May 15	-17
1921	44.5	52.6	May 14	-10	May 14	-11	May 14	-11	June 3	+2	May 14	-18
1922	40.0	52.4	June 3	+10	May 27	+2	May 27	+2	June 9	+8	June 3	+2
1923	44.0	47.5	June 9	+16	June 2	+8	June 2	+8	June 6	+5	June 9	+8
1925	41.7	51.0	June 6	+13	June 6	+12	May 25	0	June 5	+4	June 20*	+15.5
1926	43.5	49.3	April 24	-30	May 22	-3	June 5	+11	June 5	...	June 5	+4
1927	44.7	48.5	June 4	+11	June 4	+10	May 21	-4	...	...	June 4	+3
1928	41.2	49.1	May 19	-5	May 26	+1	May 26	+1	June 1*	...	June 4	...
1929	44.8	51.3	May 18	-6	June 1*	+3.5	June 1*	+3.5	June 1*	-3.5	...	...

	<i>S. REFLEXA</i>		<i>S. YUNNANENSIS</i>		<i>S. ADAMIANA</i>		<i>S. EMODII</i>		<i>S. EMODII VARIEGATA</i>		AVERAGE OF B COLUMNS
	A	B	A	B	A	B	A	B	A	B	
1919	May 29	-8	June 14	+4	June 14	+2	June 7	-10	June 14	-11	-2.25
1920	...	...	May 22	-19	May 22	-21	May 29	-19	June 19	-6	-13.5
1921	...	...	June 4	-6	June 11	-1	June 4	-13	June 11	-14	-9.0
1922	June 10	+4	June 10	0	June 3	-9	June 24	+7	July 8	+13	+3.25
1923	June 9	+3	June 23	+13	June 30	+18	July 7	+20	June 30	+5	+10.75
1925	June 20*	+10.5	June 20*	+6.5	June 20*	+4.5	July 4*	+13.5	July 4*	+5.5	+8.5
1926	May 22	-15	May 29	-12	June 11	-1	June 5	-12	...	...	-6.5
1927	June 11	+5	June 11	+1	June 11	-1	July 7*	+16.5	July 2	+7	+4.0
1928	June 16	+10	June 16	+6	June 16	+4	...	...	July 7*	+8.5	+5.25
1929	June 1*	-8.5	June 22†	...	...	...	...	...	June 22†	...	-2.5

\* Record made at end of a fortnight.

† Record made at end of three weeks.

The composite graph of these ten later-flowering species (fig. 8, S2c) corresponds with the curve of March temperature (S2a); that is, of the proximal belt, except in 1923 and 1927. In 1923, March temperature was high but flowering was very late. In 1927, March temperature was also high, and flowering was again rather late. The graph of May temperature (S2b); that is, of the immediate belt, shows that the two lowest temperature records for that month occurred in the years 1923 and 1927, and may reasonably be taken to account for the deviations. In 1919, May temperature has modified but not overridden the effect of March temperature.

It must be noted that in 1926 there is indicated (fig. 8, S2c) a diversity in the behavior of the different species. With the available data it is not desirable to attempt to account for these, although the explanation offered in the case of *Cytisus* might be considered, namely, that a continuous advance of development through all the stages of spore formation exposes the plant for a longer time to action by external factors; and further, that at any "moment" in time the process will be more or less reactive to "momentary" fluctuations of the external factor. By momentary here is meant rather a short period, of one or two days at most.

### Discussion

The foregoing data seem to indicate that the species of *Rhododendron*, *Cytisus*, and *Syringa* under consideration are affected as regards their date of flowering by the temperature at certain specified times during the year. While one species may in any particular year deviate from the general behavior of the natural group into which it falls here, nevertheless when all species are considered they show over a period of ten years a significantly consistent response to the temperature of distal and proximal, or proximal and immediate, weather belts.

It would seem that lower temperatures, obtaining during these significant though sometimes remote periods, delay the opening of flower buds; and conversely higher temperatures obtaining during the same periods in other years induce earlier flowering. The comparatively narrow belts of time during which deviations of temperature may be significant in inducing these effects are believed to synchronize with activity in spore formation in the plant. In other words, higher temperatures, occurring when there is activity in spore formation, lead to earlier flower burst; and lower temperatures, occurring at the same point, lead to later flower burst. The detailed mechanism through which such an effect may be brought about is deep seated and obscure. It would seem adequate simply to regard the higher temperatures as expediting the reactions involved in meiotic and pre-meiotic cell division; and there is no doubt that this must in large part be true.

It is a matter of common observation and frequent record that different species of plants develop generally in definitely different temperature ranges, some plants requiring for normal growth an environment with a higher temperature range than others. Again some plants are more particular and demand a narrower range of temperature; and exposure to temperature below a minimum, higher than the minimum of other more "hardy" species, inhibits development and even causes death. These specific demands are, as has been said, well known although not well understood, and may be taken as the factor which conditions the "average" flowering date of different species.

Furthermore, the temperature demands of different organs of any one plant are not the same. In the great majority of cases in the annual history of the flowering plant vegetative activity occurs first in time, and usually at temperatures lower than will occur subsequently. The vegetative activity is followed by the change to reproductive phase, the change of phase being synchronized with onset of higher temperatures, so that in a scale of rising temperatures the reproductive phase comes as a culmination to the activity of the plant. Reverse cases are known, as for example *Tussilago farfara*, in which the reproductive phase is synchronized with the earlier period of lower temperatures, and flowering precedes vegetative activity. It may be that in the onset of the reproductive phase in such a genus as *Rhododendron*, where microspore and megaspore development take place in summer and spring respectively, this activity is dependent on temperature of narrow range, and may be inhibited, especially in the earlier flowering species, by higher as well as by lower temperatures.

While stress has been laid here on the connection between temperature belts and spore formation, it is not overlooked that the metabolic complex culminating in general development may be the fundamental cause of the fluctuations. It is significant that the localization in time has been related to a definite step in development.

### Summary

Analyses of records on the date of flowering of various members of different genera at the Royal Botanic Garden, Edinburgh, show the following points:

1. The actual date of flowering of any species in any one year may vary from the average date.
2. Such aberration is referable to the temperatures obtaining during narrow belts of time.
3. These belts of time vary between genera as to the remoteness from the actual date of flowering, and here are referred to the periods of activity in gamete formation.

The writer is greatly indebted to Dr. ALEX. NELSON, Edinburgh University, for much help and encouragement during the course of the work, and to Professor W. WRIGHT SMITH and the staff of the Royal Botanic Garden, Edinburgh, for permission to use the data, and for other facilities.

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# EFFECT OF FREQUENT CLIPPING ON THE DEVELOPMENT OF CERTAIN GRASS SEEDLINGS

JOSEPH H. ROBERTSON

(WITH TEN FIGURES)

## Introduction

The study reported here deals with the effects of the removal of tops upon the development of various species of grass seedlings. Not only the establishment but also the yield and nutritive value of range and pasture grasses are dependent to a great extent upon the manner and frequency with which the tops are removed. Winter-killing and lowered vitality, with attendant decreased yield, result from improper utilization of the herbage. In native pastures and range lands, undesirable species increase when there is early grazing and overstocking (8). Although the relation of harvesting practices to the development of grassland is known in a general way from observation, and more definitely for a few species from experiment, there is still a definite need for further experimental work.

GRABER (7) has shown that frequent removal of tops limits root development. This in turn may lessen drought resistance and absorptive capacity, and result in increased injury from low temperature and insects. Since most of the foods used as reserves in roots are synthesized above ground, frequent harvesting necessarily limits these reserves. Internal food reserves as well as a supply of nutrients are factors in productivity. In addition, frequent removal of tops promotes a deficiency of nitrogen in the soil (7).

Dicotyledonous plants are similarly weakened by too early or too frequent harvesting. Pruning reduces the root spread of almond trees (3). Cutting of immature alfalfa increases invasion of weeds and may cause winter-killing. The specific gravity of alfalfa roots decreases slightly after each cutting. Cutting when the plants are mature gives more reserve food and higher yield with less winter-killing and a longer life of crop, whether grass or alfalfa (1). SAMPSON and MALMSTEN (16) found that harvesting range grasses twice each season increased the yield with but slight injury to the roots. Yield tends to vary inversely with frequency of clipping in bluestem pastures of Kansas. Disappearance of valuable species is proportional to frequency of cutting. The higher nutritive value gained from frequent harvesting does not compensate for loss in yield (2). The deleterious effect of frequent cutting may be offset partially by cutting the plants at a greater height above the ground (2, 9, 18). Reseeding of western range land is successful only when grazing is delayed until late in the season.

WOODMAN, NORMAN, and FRENCH (20) found that the nutritive value of pasture herbage, under fertilization and abundant rainfall, is independent of cutting periods. Increased carrying capacity, under rotational grazing as compared with continuous grazing, was in the ratio of 3:2.

Many of the trials made recently show that the behavior of grasses following clipping is largely influenced by diminished food reserves and retarded development of roots. Normal root growth is rhythmic and, on the basis of dry weight, may be plotted as a series of SACHS grand-period-of-growth curves (15). This rhythm is disturbed on removal of tops.

Any dwarfing in the root system of a seedling may be regarded as serious. When the water relation is controlling, the fate of a seedling often depends upon its ability to develop an adequate root system. Under competition an added inch of growth underground may enable the plant to overtop its neighbors, the result often being the difference between survival and death. Extent of roots, rate of growth, and degree of tillering all are closely correlated (19). Seedlings of grasses are susceptible to injury from drought, erosion, and trampling, and their resistance to these is due in a great measure to the development of an adequate root system. Plants with larger root systems, either in soil or water cultures, produce more tillers upon addition of nitrogen than do those with smaller roots (6). JACKSON (10) found that nodal roots are always present at the beginning of tillering in barley. In general, higher yielding species of grasses have heavier root systems (17).

There is almost unanimous agreement that the best development of roots and tops goes hand in hand. An exceptional finding is that of LAIRD (12), who states that "the largest and deepest root systems of sod-forming grasses are not necessarily associated with the best and most vigorous top growth. Mowing of Centipede and Bermuda grasses increased the root growth" as measured by dry weight. STAPLEDON and BEDDOWS (17) compared the yield of normal plants with the bulk of their individual root systems and found no definite correlation.

According to PARKER and SAMPSON (13), frequent cutting produces measurable effects upon anatomical structure. Diameters of both root and stele as well as the number of ducts were decreased as a result of clipping. Similar results have recently been obtained by BISWELL (4).

### Experimentation

The seeds of four native prairie grasses were used. They were bluegrass (*Poa pratensis*), blue grama grass (*Bouteloua gracilis*), June grass (*Koeleria cristata*), and needle grass (*Stipa spartea*). The seeds were collected at maturity near Lincoln, Nebraska, in 1931. Seeds of Hungarian brome grass (*Bromus inermis*) and Sudan grass (*Holcus sorghum sudanensis*), both exotic pasture grasses, were purchased at a local seed store.

The soil used was fertile loess having a hygroscopic coefficient of 8.9 per cent., a humus content of 7.6 per cent., and a circumneutral reaction. After screening the soil, sufficient water was added to increase its moisture content to about 30 per cent., which was approximately optimum for growth.

Forty-eight galvanized iron containers were used, ranging from 8 to 24 inches in depth and from 16 to 72 square inches in cross-sectional area. They were filled on October 16, the soil being uniformly compacted in all. Seeds of each of the six species of grasses were then planted, eight containers being used for each species. Four containers for each species were labeled 1, 2, 3, and 4, and the four remaining ones 1A, 2A, etc. The shallower containers were used for the plants that were to be grown only a few weeks; the deeper ones for the longer periods of growth. Thus the plants had, with few exceptions, ample room for normal depth of root development. The weight of each filled container was recorded.

With the exception of those with bluegrass, the containers were kept covered for six days following planting. Light sprinkling for a period of ten days insured rapid germination. Seven days after planting, the containers were restored to the original weight by adding water, and the heights of the seedlings were measured. This was repeated every seventh day during the experiment. The containers were also turned and shifted at these times to insure equal reception of light.

When a good stand was assured, brome grass and grama grass were thinned to 25 plants per container; bluegrass and June grass to 50 plants; and the coarser Sudan grass and needle grass to 12 plants.

Since the work was done during winter, sunlight was supplemented by two 150-watt electric lights. They were mounted in 15-inch white enameled reflectors suspended 3 feet above the tops of the containers. These lights were employed from 5:30 P. M. to 8:00 P. M., October 23 to February 18. In addition, they were used during cloudy weather. Measurements of light intensity, taken on January 17 at 12:00 M. with a Weston photronic cell and milliammeter, gave a ratio for outdoor direct sunlight to sunlight in the greenhouse of 7:6. The ratio for artificial light at 8:00 P. M. to sunlight at noon in the greenhouse was 1:15 directly under the reflector and 1:90 at the tops of the containers farthest from the source of light.

It was decided that under the conditions of this experiment, grazing could be represented best by removing the tops of the grasses by clipping. Accordingly, when each species reached a height suitable for grazing, the plants in containers labeled 1A, 2A, etc., were clipped. Sudan grass was clipped 3 cm. above the soil surface and the other grasses 1.5 cm. The tops were placed in weighing bottles and dried in an electric oven for 24

hours at 75° C. After being cooled in a desiccator they were weighed to the nearest 0.01 gm.

When the clipped plants had again reached sufficient height for grazing, tillers were counted and the tops again removed and weighed. This was also done with the unclipped plants of each species in containers no. 1. The soil was then carefully washed away from the roots in containers 1 and 1A. This was accomplished by placing the containers in a slanting trough and gently playing a small stream of water on the soil. The escaping muddy water was run through a small-meshed sieve and the few root ends that were broken off were recovered. These were included in the measurements of weight and volume.

Comparisons of roots included average length, volume, dry weight, and anatomical structure. Volume was found by displacement of water in a cylindrical graduate after the water on the surface of the roots had been removed by pressing them between paper towels. Portions of adventitious roots, 2.5 to 4 cm. from their tips, were fixed in chromo-acetic acid solution. Transverse sections of these were cut 15  $\mu$  thick and compared as to internal structure.

Individual root systems of Sudan grass and needle grass were separated from the mass of roots after they were placed in the shallow pans and floated in water. Root systems of average size were selected. Black paper, against which the light-colored roots showed plainly, was cross-ruled with white lines and fastened in the bottom of the pan, which was then covered with 1.5 inches of water. The root systems were floated out to their natural positions and drawn life-size on cross-ruled paper.

When the clipped plants had again reached their former height, the tops were clipped a third time and comparisons of roots and shoots of plants in containers 2 and 2A were made. This process was continued until the last lot of plants was examined on February 26.

Because of differences in rate of growth and in ability to recover after clipping, as well as variable periods of cloudiness, it was necessary to vary the length of intervals between clippings.

The temperature of the greenhouse averaged 23° C. but varied from 15° to 30° during the eighteen weeks of the experiment. The humidity at all times was rather high. Soil samples taken from containers showed that the variation in moisture content was between 21.6 and 30.4 per cent.

An attack of aphids made it necessary to spray the plants on January 15. The Sudan grass was injured to some extent by the sulphur-tobacco mixture.

## Results

### *BOUTELOUA GRACILIS*

The grama grass was clipped when five weeks old and at 21-day intervals thereafter. At the time of the first clipping it was 8 cm. tall and had

three leaves per plant. Thus it had made an average daily growth, after appearing above ground, of 2.6 mm.

A comparison of growth rates after each clipping shows that the first and third treatments inhibited elongation slightly while the second and fourth stimulated it somewhat. The total result of the four clippings was a slight inhibition. The total increase in height during the seventeen weeks was 23 cm. for the clipped and 24 cm. for the unclipped plants.

Six leaves were produced by the control and four by the clipped plants during the period of the experiment. Tillers were ten times as numerous and sixteen times as tall on the unclipped as on the clipped plants (table I).

Dry weight of tops decreased after the first cutting and increased slightly after each of the last three cuttings (fig. 1). The initial decrease may have been due to shortening days, November 20 to December 11, coincident with 73 per cent. cloudiness. The total dry weight of the clipped tops was 0.15 gm. The tops of the control plants weighed 2.46 gm., or over sixteen times as much.

A marked difference in growth of roots was found. While the roots of the control plants penetrated continuously to a depth of 33 cm., those of the clipped plants decreased with each removal of tops (fig. 1). Neither the dry weight nor the volume of the roots increased markedly after the first clipping. The final dry weight ratio was 1:35 for roots of clipped and unclipped plants respectively. That of volume was 1:52 (table I).

The diameters of the roots of clipped and unclipped plants were in the ratio of 3:4. Fifty per cent. of the diameter of the clipped plants consisted of stele while in the unclipped ones the stele composed only 46 per cent. Xylem strands were more abundant in the latter (table II).

Table I gives the percentage of survival after each of the cutting treatments.

#### *BROMUS INERMIS*

Clipping was begun 28 days after planting, when the seedlings were 13 cm. tall and in the third-leaf stage. They had grown at the rate of 5 mm. per day after germination. During the 13 weeks the clipped plants elongated 10 cm. more than the unclipped ones. Thus clipping resulted in a 23 per cent. increase in height.

At the end of the experiment the undisturbed plants had eight leaves while those clipped four times had five. The clipped plants did not tiller. An average of six tillers 25 cm. tall was produced by the control plants (table I). The final leaf widths were 2.4 mm. and 5.8 mm. for the clipped and unclipped plants respectively.

Weight of tops decreased after each clipping (fig. 2). The five separate cuttings yielded 4 per cent. as much dry weight as the one final cutting.

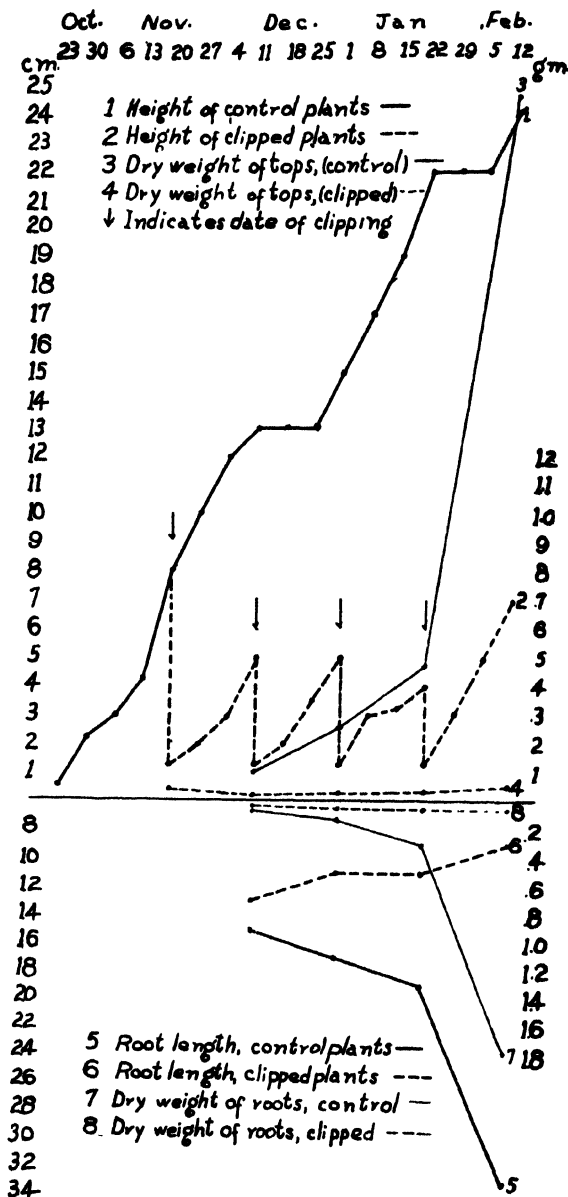


FIG. 1. Growth of roots and tops of *Bouteloua gracilis*.

TABLE I  
DATA ON TOP AND ROOT DEVELOPMENT NOT INCLUDED IN FIGURES 1-10. FIGURES BASED ON CONTAINERS REMOVED ON DATES INDICATED

SPECIES	DATE OF CLIPPING	NUMBER OF LEAVES		NUMBER		TILLERS		ROOT VOLUMES		SURVIVAL PERCENTAGE	
		UNCUT	CUT	UNCUT	CUT	UNCUT	CUT	UNCUT	CUT	UNCUT	CUT
							HEIGHT				
							cm.		cc.		%
<i>Bouteloua gracilis</i>	Dec. 11	5.0	2.5	0.0	0.0	0.0	0.0	0.3	0.2	100	100
	Jan. 1	5.0	3.6	0.7	0.0	4.0	0.0	0.5	0.1	100	80
	Jan. 22	5.0	3.0	2.1	0.2	8.0	2.0	1.5	0.3	100	72
	Feb. 12	6.0	4.0	5.0	0.5	16.0	1.0	10.5	0.2	100	92
<i>Bromus inermis</i>	Dec. 2	6.0	4.0	0.5	0.1	1.3	0.2	5.5	2.3	100	100
	Dec. 18	6.0	5.0	0.5	0.0	8.0	0.0	13.0	1.8	100	100
	Jan. 3	7.0	5.0	2.5	0.0	4.0	0.0	23.0	1.7	100	100
	Jan. 18	8.0(2)*	5.0	6.3	0.0	25.0	0.0	56.0	0.8	100	68
<i>Holcus sorghum sudanensis</i>	Dec. 2	5.0	3.0	2.0	0.2	5.0	3.0	5.0	1.1	100	100
	Dec. 18	5.5	3.0	2.8	2.0	11.0	5.5	11.6	1.4	100	83
	Jan. 3	8.0	3.0	6.0	1.0	31.0	7.0	43.5	0.8	100	83
	Jan. 18	8.0(3)	3.0	11.0	1.5	41.0	6.0	62.0	1.5	100	75
<i>Koeleria cristata</i>	Dec. 23	5.5	4.0	0.8	0.2	2.0	0.5	1.2	0.3	100	100
	Jan. 11	6.0	4.0	1.0	0.5	1.5	0.4	4.8	0.7	100	100
	Jan. 30	6.0(2)	4.0	3.0	0.1	7.0	1.0	3.7	0.9	100	100
	Feb. 20	7.0(2)	5.0	2.5	1.4	8.0	3.0	8.0	2.9	100	100
<i>Poa pratensis</i>	Dec. 9	6.0	3.0	1.0	0.0	5.0	0.0	13.0	8.5	100	100
	Dec. 23	6.0	3.0	1.5	0.0	2.0	0.0	29.0	11.0	100	100
	Jan. 11	7.0	3.0	1.1	0.0	11.5	0.0	25.0	15.0	100	100
<i>Stipa spartea</i>	Dec. 9	3.0	2.0	0.0	0.0	0.0	0.0	2.1	1.6	100	100
	Jan. 6	5.0(1)	2.0	0.2	0.0	15.0	0.0	2.5	0.4	100	65
	Feb. 3	5.0(1)	2.0	2.0	0.0	23.0	0.0	15.3	0.7	100	75
	Feb. 26	5.0(1)	2.0	3.0	0.0	30.0	0.0	20.5	1.1	100	75

\* Figures in parentheses indicate number of dead leaves.

TABLE II

EFFECT OF FOUR CLIPPINGS ON ANATOMICAL STRUCTURE OF NODAL ROOTS. DATA BASED ON TRANSVERSE SECTIONS  $15\ \mu$  THICK, FROM PORTIONS OF ROOTS 2.5 TO 4 CM. FROM TIPS

SPECIES	TREATMENT	DIAMETER OF ROOT	DIAMETER OF STELE
		$\mu$	$\mu$
<i>Bouteloua gracilis</i>	{ Clipped	252	126
	{ Unclipped	332	151
<i>Bromus inermis</i>	{ Clipped	189	113
	{ Unclipped	490	215
<i>Holcus sorghum</i>	{ Clipped	248	126
	{ Unclipped	549	312
<i>Koeleria cristata</i>	{ Clipped	262	119
	{ Unclipped	216	91
<i>Poa pratensis</i> *	{ Clipped	119	66
	{ Unclipped	329	112
<i>Stipa spartea</i>	{ Clipped	307	134
	{ Unclipped	472	168

\* After two clippings.

Root penetration was practically stopped by clipping. Increase in length was slow until the fourth cutting, which was followed by a slight decrease. The unclipped plants had roots which were nearly four times as long as those of the clipped ones (figs. 2, 3). The ratio for the dry weight of roots was 1:51 for the clipped and unclipped plants respectively. The corresponding volume ratio was 1:70.

A response of brome grass to removal of tops was observed by growing seedlings in soil in glass jars 2 feet deep. These were tilted at an angle of  $20^\circ$  from the vertical. Rate of growth down the side of the jar was observed to diminish gradually as a result of clipping. Growth then ceased entirely for 12 days before the roots started to die back from the tips. The rate at which they died back was approximately the same as that at which they had penetrated before clipping was begun.

The diameters of the roots of cut and uncut plants were in the ratio of 3:8. The stele of the treated plants made up 59 per cent. of the diameter as compared with 43 per cent. for the untreated ones. Metaxylem elements were larger and more than twice as numerous in the uncut plants.

Survival fell to 68 per cent. after the fourth cutting (table I).

#### *HOLCUS SORGHUM SUDANENSIS*

The seedlings of the Sudan grass were first clipped 4 weeks after planting. At that time they were 13 cm. tall and in the late fourth-leaf stage.

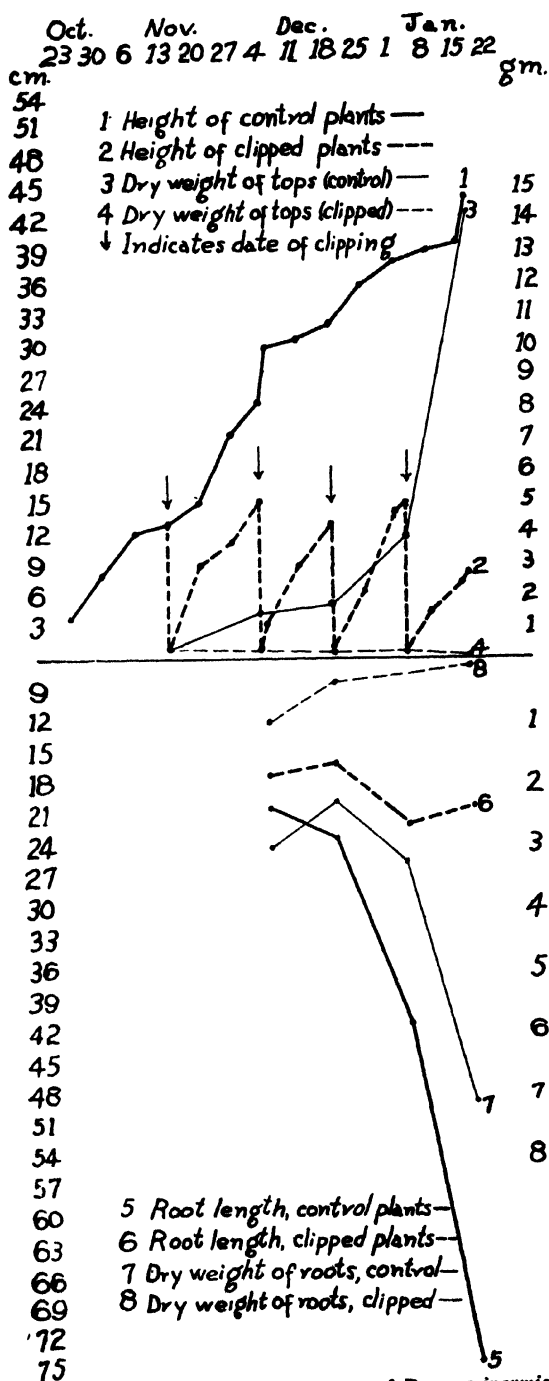


FIG. 2. Growth of roots and tops of *Bromus inermis*.



FIG. 3. Unclipped and clipped *Holcus* (left) and *Bromus* (right). 1/11 natural size.

After every clipping the unclipped plants elongated more rapidly than the clipped ones. During nearly half of the experiment the rate of elongation of the cut plants was less than one-half as rapid as that of the controls. The total increase in height of the clipped plants was 39.5 cm.; the final height of the unclipped ones was 60 cm.

By the end of the 13 weeks, the unclipped plants had eight leaves but the clipped ones only three. Tillers were over seven times as numerous and nearly seven times as tall on the unclipped as on the clipped ones (table I).

Dry weight of tops decreased after the first and second clippings but increased slightly after the third and fourth, probably as a result of difference in length of day (fig. 4). The ratio of total dry weight was 1:14 for clipped and unclipped plants respectively. The final leaf widths were 6.1 mm. on the clipped and 13.6 mm. on the unclipped plants.

Differences in length of roots increased after each treatment. The roots of the clipped plants increased in length until the fourth clipping and then decreased (fig. 4). Sixteen days after the first clipping, the roots of the clipped plants were three-fifths as long as those of the unclipped. At the same length of time after the fourth cutting, the ratio was 3:7. The dry weight ratio at the end of the experiment was 1:40 and the volume ratio 1:41 for the clipped and unclipped plants respectively.

The clipped plants were characterized by short adventitious roots and nearly normal primary roots (figs. 5, 6). The adventitious roots began to die back from the tips after the second clipping. The plants apparently maintained a certain balance between development of roots and tops. Production of tillers and growth of adventitious roots correlated closely (table I; figs. 5, 6).

Roots of the control plants were more than twice as great in diameter as those of the clipped ones. Fifty-one per cent. of the diameter of the latter was stele, as was 57 per cent. of the diameter of the roots of the control plants (table II).

Survival fell to 83 per cent. after the second clipping and to 75 per cent. after the fourth (table I).

#### *KOELERIA CRISTATA*

On December 4 the seedlings of the June grass had four leaves each and were 6.5 cm. tall. This amounted to 1.4 mm. growth daily since germination. The plants were clipped at this date and four times thereafter at intervals of 21 days. The tops of the clipped plants elongated faster than those of the unclipped throughout the period of the experiment. That the growth of these plants was stimulated by cutting is shown further by the fact that the control plants attained a height of only 15.5 cm. while the total increment of the clipped ones was 19.5 cm.

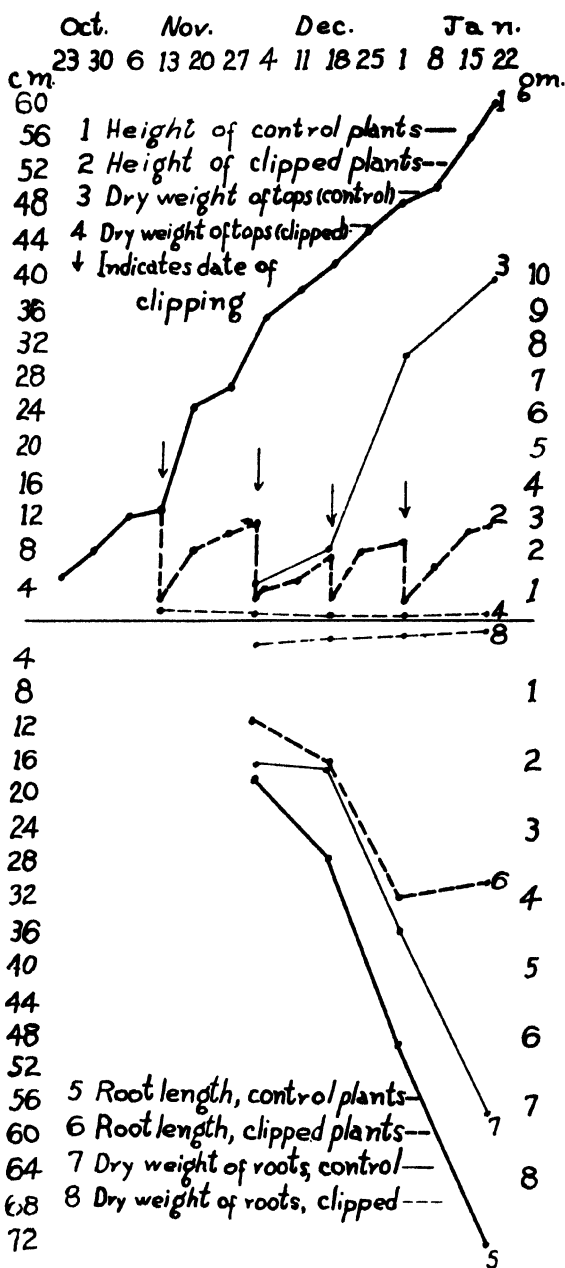


FIG. 4. Growth of roots and tops of *Holcus sorghum sudanensis*.

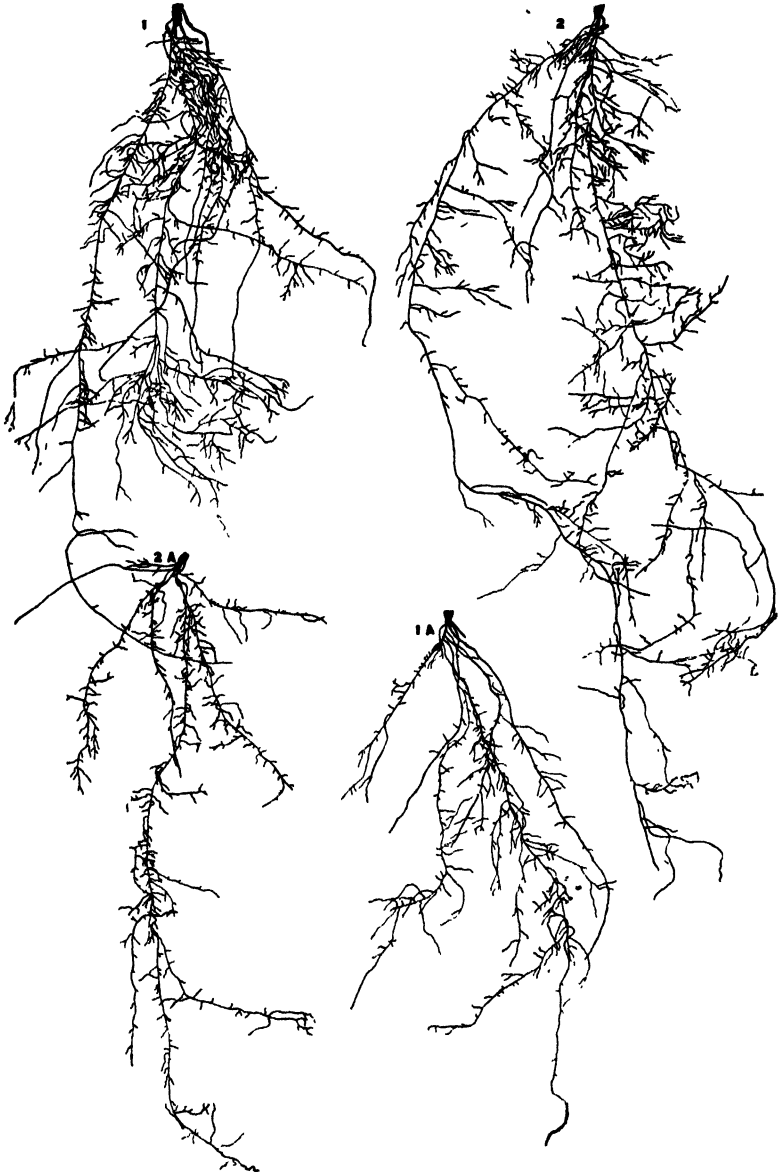


FIG. 5. Roots of *Holcus sorghum sudanensis*: 1, unclipped; 1A, clipped once (both 7 weeks old); 2, unclipped; 2A, clipped twice (both 9 weeks old). About  $\frac{1}{4}$  natural size.

The plants clipped four times had an average of five leaves; those not cut had seven. Tillers were nearly twice as numerous on the control plants and nearly three times as tall as on the clipped ones (table I).

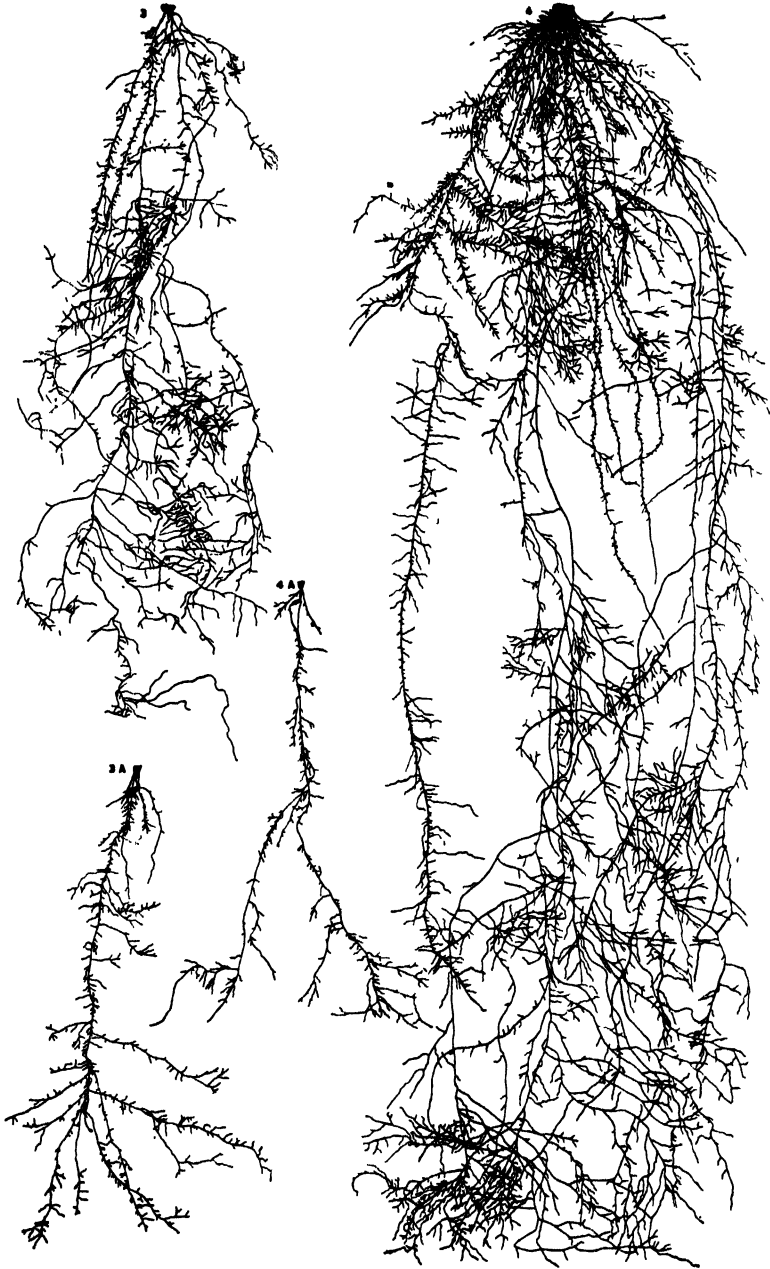


FIG. 6. Roots of *Holcus sorghum sudanensis*: 3, unclipped; 3A, clipped three times (both 11 weeks old); 4, unclipped; 4A, clipped four times (both 13 weeks old). About  $\frac{1}{2}$  natural size.

Each of the first four cuttings yielded approximately the same weight of dry matter. The fifth cutting showed considerable increase (fig. 7). Evidently the interval was sufficient to permit recovery as the days lengthened from January 30 to February 20. The five cuttings yielded nearly one-fifth as much as the one final cutting, which was much greater than for any of the other clipped grasses. This correlated with the relatively small differences in number and width of leaves. The number of living leaves (five) was the same in each at the end of the experiment. Those of the control plants were 1.6 mm. wide; the clipped ones were 1.5 mm. in width.

Root penetration continued in spite of clipping (fig. 7). The cutting treatment retarded growth an average of only 1.0 mm. per day throughout the experiment. The effect of clipping becomes more apparent when the weights of the roots are compared (fig. 7). Clipping reduced the weight of roots to one-third that of the control. The corresponding volume ratio after 18 weeks was 10:36.

Unlike the other species, the June grass responded to clipping by producing roots of greater diameter, the ratio being 6:5 for cut and uncut plants (table II). All of the plants survived the treatment (table I).

#### *POA PRATENSIS*

At the time of the first clipping, November 20, the bluegrass seedlings were 9 cm. high and in the fourth-leaf stage. The rate of growth, as measured in mm. per day, was reduced to a slightly different extent by each clipping treatment. On an average, clipping inhibited growth 0.5 mm. per day.

The clipping treatment permitted the formation of only three leaves while the control plants developed seven. Those of the clipped plants were 1 mm. wide as compared with 1.8 mm. for the unclipped ones. Clipping prevented tiller production (table I).

Weight of tops decreased until the third clipping, after which it increased (fig. 8). The tops produced by the control plants during the 12 weeks weighed 7.6 times as much as those of the clipped ones.

The roots made a definite response to each removal of photosynthetic area. Nineteen days after the first clipping, the weights of the roots of clipped and control plants were in the ratio of 1:4. This ratio was reduced to 1:11 by the second and 1:14 by the third treatment.

Root penetration continued in spite of clipping but was less rapid than in the control plants (fig. 8). The roots of the seedlings which were clipped three times were only three-fifths as long as those of the unclipped ones. Stated differently, clipping inhibited root penetration an average of 2 mm. per day.

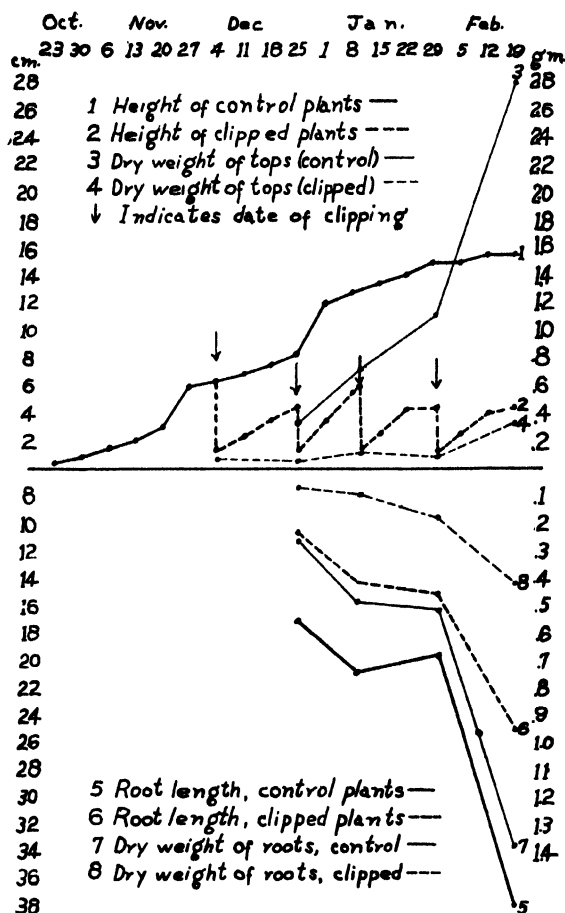


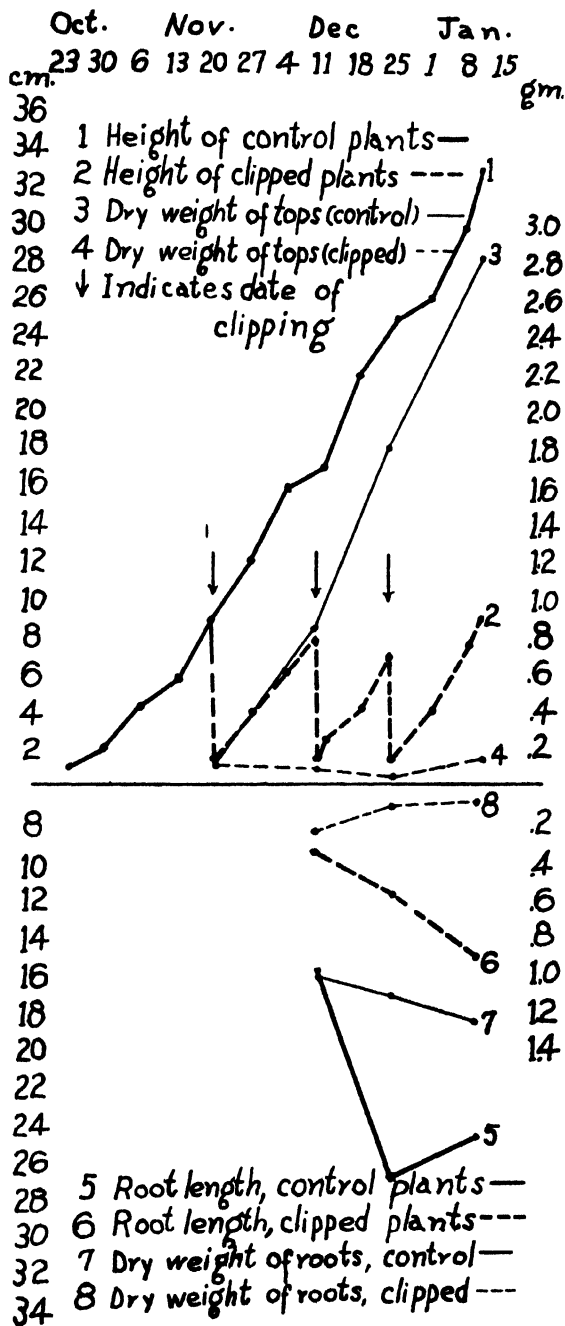
FIG. 7. Growth of roots and tops of *Koeleria cristata*.

The ratios of root volumes of clipped and unclipped plants became smaller with each treatment. They were 1:7, 1:15, and 1:20 after the first, second, and third cuttings.

As a result of two clippings, the diameter of the adventitious roots was reduced to about one-third that of the uncut plants. In the latter the stele formed 34 per cent. of the root diameter, while in the cut plants it constituted 55 per cent. (table II).

#### *STIPA SPARTEA*

The seedlings of needle grass were first clipped on November 20. On this date they had three leaves and were 12 cm. in height. The first and

FIG. 8. Growth of roots and tops of *Poa pratensis*.

second clippings apparently inhibited elongation of tops. After the third treatment no difference in rates of growth due to clipping was detected. The controls elongated only 28 per cent. as much as the cut plants after the fourth treatment. Hence clipping apparently caused a 30 per cent. increase in height.

Cutting limited the number of leaves to two while the control plants developed five. The control plants produced three tillers 30 cm. tall. Clipping entirely prevented the production of tillers (table I).

Weight of tops decreased slightly after the first and second cuttings and a small increase was evident after the third and fourth (fig. 9). These increases were probably due to the greater length of day, January 6 to February 26. The five cuttings yielded less than 7 per cent. as much dry weight of tops as one final cutting. Leaves of the clipped plants were only 0.92 mm. wide as compared with 2.53 mm. for those of the control plants.

The effect of each clipping was strongly reflected by the roots. As in the case of Sudan grass, the chief difference was in the checked development of adventitious roots (fig. 10). Development of these roots was prevented by the first three cuttings. Their growth after the fourth cutting was evidence of recovery. With but one exception, root penetration continued after each clipping; but the weight decreased until the fourth clipping, after which a slight increase was made (fig. 9). At the conclusion of the experiment the control plants had roots which were 2.6 times as long as those of the clipped ones. Clipping was thus responsible for retarding root penetration to an average extent of 2.7 mm. daily during the 130 days.

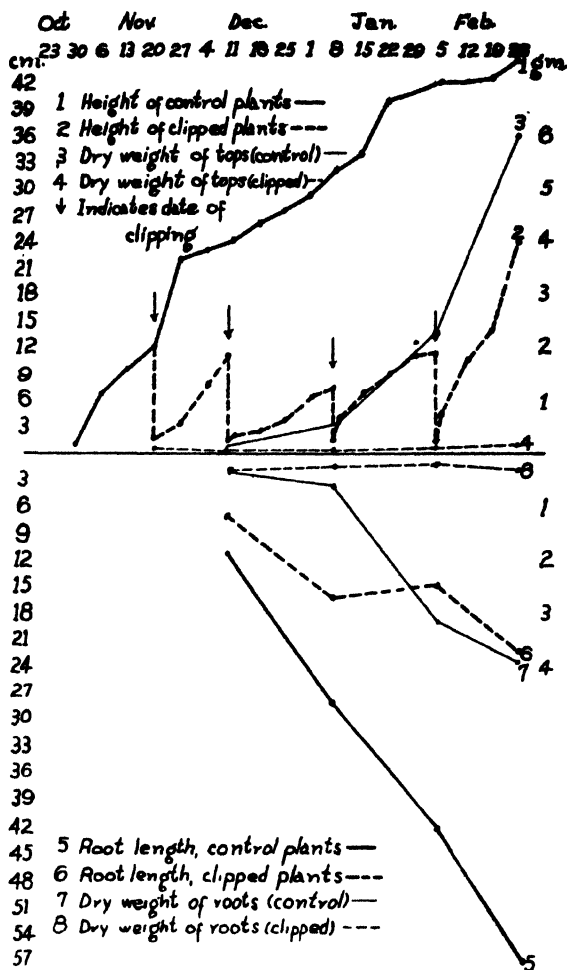
The volume ratio of the roots of unclipped plants to those of plants clipped four times was 18:1. The corresponding weight ratio was 13:1.

The roots of the clipped plants were only two-thirds the diameter of those of the unclipped ones. In the clipped plants the stele made up 44 per cent. of the root diameter, while in the control plants it composed only 35 per cent.

Seventy-five per cent. of the plants survived the four treatments.

### Discussion

This experiment shows clearly that the development of grass seedlings is seriously affected by frequent removal of tops. Certain species, such as *Koeleria*, are injured much less than others, such as *Stipa* and *Holcus*. GRABER (7) reports that nine cuttings injured alfalfa more than twenty-two cuttings did bluegrass. Differences in response are shown also by the fact that elongation of tops in three of the grasses (*Poa*, *Bouteloua*, and *Holcus*) was inhibited, while in the other three (*Bromus*, *Koeleria*, and *Stipa*) it was slightly stimulated by clipping.

FIG. 9. Growth of roots and tops of *Stipa spartea*.

All of the species responded to clipping by reduction in yield and decreased development of roots. This was effected through reduction in number and height of tillers, number and width of leaves, and number and length of roots. The close relation in *Stipa* and *Holcus* between the production of tillers and nodal roots is in accord with the finding of KRASSOVSKY (11) for wheat and barley.

The nodal roots were the underground parts chiefly affected. In *Stipa* these roots were not produced until after the fourth cutting of tops. The tops also showed marked recovery at this time. The nodal roots of *Holcus* were produced early in the experiment and were reduced gradually by the

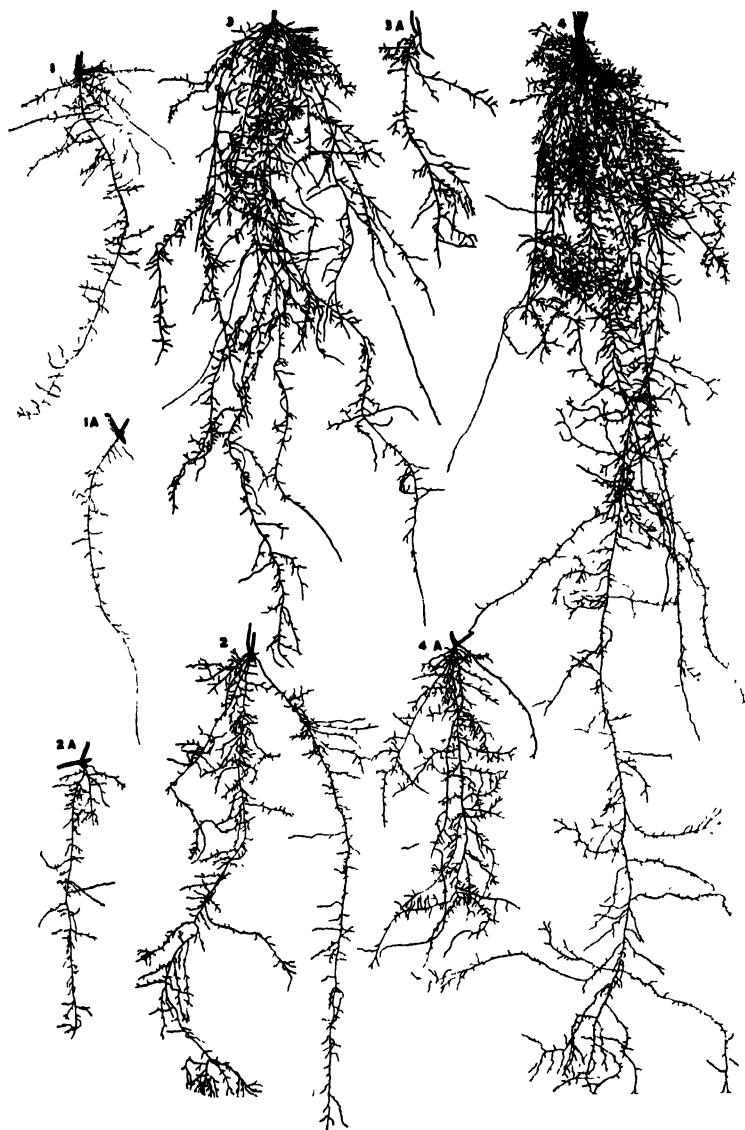


FIG. 10. Roots of *Stipa spartea*: 1, unclipped; 1A, clipped once (both 8 weeks old); 2, unclipped; 2A, clipped twice (both 12 weeks old); 3, unclipped; 3A, clipped three times (both 16 weeks old); 4, unclipped; 4A, clipped four times (both 19 weeks old). Slightly less than  $\frac{1}{2}$  natural size.

clipping treatment. Clipping retarded the roots of *Bouteloua* to one-fifth the length of the controls. Roots of *Koeleria* reached about five-eighths their normal length.

The mass of the roots, based on dry weight, decreased or remained constant except for *Koeleria*, which increased in spite of clipping. *Bromus* suffered the greatest reduction in both tops and roots on the basis of dry weight. The tops of the clipped plants weighed one-twenty-fifth and their roots only one-fiftieth as much as those of the controls. The corresponding ratios for *Holcus* were 1:14 and 1:40. In *Stipa* the reduction of tops was approximately the same as that of roots, being 1:14 and 1:13. The roots of the clipped *Poa* were one-fourteenth and the tops one-eighth as heavy as those of the unclipped plants. *Koeleria* was injured the least as measured by reduction in weight of tops and roots.

In spite of clipping, penetration continued slowly in all species except *Bouteloua*, which responded by a gradual decrease in root length. It is significant that the roots of *Bromus* ceased growth for 12 days before starting to die back from the tips following repeated removal of tops. PARKER and SAMPSON (14) report a 15-day cessation of growth in 15-day-old *Bromus hordeaceus* after one clipping. Evidently there is more or less of a balance between the root system and the photosynthetic area necessary to sustain it.

All of the grasses except *Koeleria* produced roots of greater diameter when the tops were not cut. In all cases except *Holcus* the stele of the roots of the clipped plants formed a higher percentage of the root diameter than those of the unclipped. This suggests that the growth of cortex is limited more by scant food supply than is that of stele. In every case the greater number of xylem strands occurred in the larger steles.

Several of the grasses showed marked differences in ability to recover after clipping. *Koeleria* showed no losses, while survival of *Stipa* fell to 65 per cent. in one instance. Under the conditions of the experiment, *Koeleria* was least injured by clipping. This may be due to its slow growth and to the fact that so much of its green tissue is near the soil surface. As measured by reduction in total weight, *Holcus* and *Bromus* were injured most severely. Seedlings are apparently injured to a somewhat greater extent by clipping than are well established grasses. BISWELL (4) reports that frequent clipping of grasses grown from sod reduced yield to one-fourth and root weight to one-tenth that of the controls.

Unless it is necessary to conserve soil moisture, as in the case of winter wheat (5), grass seedlings should be protected against grazing in order to obtain maximum development of roots and foliage.

### Summary

1. Seedlings of six range and pasture grasses were grown in soil in the greenhouse and their development was studied after each of four clipping treatments.

2. Growth of tops, as measured by dry weight, decreased owing to clipping. Reduction in yield ranged from 80 to 96 per cent. for the different species. Elongation of tops was stimulated in one-half the species and inhibited in the other half. Width and number of leaves and number of tillers were reduced by clipping.

3. Removal of tops invariably retarded root penetration but seldom stopped it completely. Nodal roots were the underground parts affected most. In most cases these roots were nearly absent after the fourth clipping, either having failed to develop or having died back from the tips. Excepting in *Koeleria*, the nodal roots of the clipped grasses were of smaller diameter and had relatively larger steles than those of the unclipped plants.

4. The greatest reduction in yield, that of *Bromus*, accompanied the greatest retardation in root development. Minimum reduction in both root and shoot occurred in *Koeleria*. In general, clipping reduced growth of roots, as based on dry weight, about twice as much as that of tops. In *Koeleria*, however, the weight of tops was reduced more than that of roots. In the six species the roots of the clipped plants ranged in weight from one-third to one-fiftieth as much as those of their controls.

5. Excepting in *Koeleria*, the dry weight of roots decreased or remained constant after each clipping. Root volumes correlated fairly well with dry weights.

6. Removal of the aerial parts of grass seedlings had an immediately injurious effect which was measurable both above and below ground. Extent of injury depended largely upon the nature of the species and the frequency of the treatment.

The writer expresses his appreciation to Dr. J. E. WEAVER, who suggested the problem and gave much helpful advice throughout the course of the research.

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# QUANTITATIVE RELATION BETWEEN CHLOROPHYLL AND IRON IN GREEN AND CHLOROTIC PEAR LEAVES

J. OSERKOWSKY

(WITH SIX FIGURES)

## I. Introduction

Lime-induced chlorosis is known to be curable by iron, and for this reason is attributed to iron deficiency. Chlorotic leaves should accordingly contain less iron than green leaves. It has been found by several investigators, however, that chlorotic leaves may contain more iron than green leaves. Similar results were obtained also by the writer, regardless of whether the iron content was reported on the dry or on the fresh-weight basis. No positive correlation could be observed between the iron content and the amount of chlorophyll of leaves obtained from chlorotic trees (table II, columns 4 and 5), although iron was the limiting factor in these leaves in so far as chlorophyll formation was concerned. This fact may be explained by either of the following hypotheses:

1. All the iron present in the leaves is equally (or nearly equally) active in chlorophyll formation. The efficiency of its activity, however, may vary considerably in leaves of the same tree. The green leaves would accordingly be those leaves which may contain small amounts of iron, but in which the efficiency of the iron in chlorophyll formation is very great.

2. Only a fraction of the iron in the leaves is active in chlorophyll formation. This active fraction is more abundant in the green leaves than in the chlorotic ones, although the reverse may be true in the case of the total amount of iron in the leaves.

In regard to the first hypothesis, it may be stated that a wide range of variations in the efficiency of the iron in leaves is logically not impossible. On the other hand, the assumption that all the iron in leaves is active in chlorophyll formation seems improbable, since not all the iron is present in one form. Thus BOUSSINGAULT (2) could extract with alcohol only about one-fourth to one-half of the iron content in leaves. SERGER (11) also found that not all the iron in spinach leaves could be extracted with alcohol, or with a mixture of benzene, chloroform, and ether. The investigations of SUZUKI (13), GRIESSMEYER (6), and INGALLS and SHIVE (7) indicate also that the iron in leaves is present in more than one form.

This evidence favors the assumption that a specific form of iron is active in chlorophyll formation. This form of iron is designated in this paper *active iron*. In the following pages, the attempts which were made to determine the amount and the nature of this active iron are discussed.

## II. Material and methods

Pear trees of two varieties were used, Hardy and Bartlett. The trees grew on soil rich in lime. Most of the samples were taken from two orchards which were badly affected by chlorosis. The trees in these orchards varied greatly in the chlorophyll content of their leaves. It was not uncommon to find individual trees which bore leaves of all shades of color, ranging from cream-yellow to deep green, and often green and chlorotic trees stood side by side.

Repeated treatments over a period of four years showed that the trees in these orchards always responded to application of iron, when applied in any of the following ways: spraying of leaves with iron salt solutions; injection of iron salt solutions into the trunk and limbs; and application of powdered iron salts into holes bored in the lower end of the trunk or branches.<sup>1</sup> Positive results were obtained regardless of the acid radical attached to the iron, provided the iron compounds were fairly soluble. The following compounds were found to induce greening of chlorotic leaves: ferric sulphate, ferric citrate, ferric chloride, ferric oxalate, ferrous sulphate, and ferrous citrate. On the other hand, application of citric acid, tartaric acid, cupric sulphate, manganese sulphate, and magnesium salts failed to give positive results. It is thus obvious that the plants dealt with in this investigation suffered from a typical lime-induced chlorosis due to a deficiency of iron or an abnormal iron metabolism.

Preliminary work had shown that failure to wash the leaves before analysis may vitiate the iron determination by more than 100 per cent. Thus all the leaves were washed well in distilled water before being analyzed. After washing, the leaves were dried at 50°–60° C., and then ground in a porcelain mortar or in a brass mill specially built for the purpose. Care was taken to avoid contact between iron and the samples.

The leaf powder was ashed in porcelain or silica crucibles, and the iron in the ash determined colorimetrically by the thiocyanate method as modified by WALKER (14). Care was taken to keep the standard and samples at about the same acidity, namely 0.25 N. The determination of iron in apricot and peach leaves, and some pear leaves, however, was carried out in 1.0 N HCl solutions. Frequent blank determinations were made with porcelain and silica crucibles, and the values for the iron content of the samples were corrected accordingly.

In the presence of small amounts of iron, for example, of 1.0 parts per million or less, in the solution to be analyzed, a modified method was employed similar to that used by STOKES and CAIN (12), the method being

<sup>1</sup> The trees were treated by Dr. J. P. BENNETT, and the writer is indebted to him for the use of the data thus obtained. For details concerning the treatments, see BENNETT (1).

based on the property of ethyl acetate and amyl alcohol to extract the red iron thiocyanate compound from aqueous solution. To one volume of the acid solution containing iron and ammonium or potassium thiocyanate, one-half to two-thirds' volume of ethyl acetate (or amyl alcohol) was added. The mixture was shaken in a separatory funnel and allowed to stand for a few minutes. The ethyl acetate was then separated from the aqueous solution and the color of the ethyl acetate solution compared with that of a standard solution treated in the same way. It was found that the ethyl acetate intensified the color, made it more stable, and was particularly suitable for the determination of small amounts of iron in the presence of small amounts of copper.

A direct contact was avoided between corks, rubber stoppers, ordinary filter paper, and the acid iron solution, since it was found that these objects may contain sufficient amounts of acid-soluble iron to vitiate the results. The solutions to be analyzed were filtered through acid-washed filter paper, and were kept in glass-stoppered flasks.

Practically all the iron values here reported represent the averages of duplicate or triplicate determinations.

When leaf material was extracted with various solvents, the following procedure was used: to about 4.7–6.6 gm. of dry powdered leaf material in a glass-stoppered flask, 50–70 cc. of solvent were added in proportion to the weight of the sample. The flasks were put in a shaker for about 24 hours.<sup>2</sup> The suspension was then centrifuged for about 20–25 minutes, and to the solid residue about 20–30 cc. of the solvent were added, mixed with a glass rod and centrifuged again for 10–15 minutes, decanted, 20–25 cc. of the solvent added once more, the solution stirred with a glass rod, centrifuged again for 10 minutes, and decanted. The decanted portions from each sample were combined, the liquid evaporated in porcelain or silica crucibles, the residue ashed, and the amount of iron in the ash determined as described.

Chlorophyll was determined always on *fresh leaves* according to the method of WILLSTÄTTER and STOLL (16, pp. 2–3). The color of potassium chlorophyllin of the samples was compared with that of a standard solution of potassium chlorophyllin prepared from pure chlorophyll isolated from fresh grass according to the method of WILLSTÄTTER and his co-workers (16, pp. 30–32).

<sup>2</sup> While it is not essential to adhere closely to this period of time (24 hours), it is very important when dealing with 1.0 N HCl extraction that the period of shaking should be the same for all samples of a given series, that is, leaves of equal age, collected from the same trees, and on the same date.

### III. Active iron in pear leaves

It was believed that in samples of leaves in which active iron was the limiting factor in chlorophyll formation, the amount of green pigments should bear a positive correlation to the amount of active iron they contain. In an attempt to isolate the active iron, green and chlorotic pear leaves of the same age were extracted with various solvents, and the amount of iron in these extracts was compared with the chlorophyll content of the samples to ascertain whether a direct relation existed between them. No such correlation was found when the leaves were extracted with distilled water or with 0.05 N HCl. Similar results were obtained also in regard to the iron in the vacuolar sap of leaves, which was secured by a method similar to that used by CHIBNALL (3): the fresh leaves were washed in water, then dried with a clean towel or filter paper, dipped in ether for 5–10 minutes, spread on filter paper to dry for 10–20 minutes, then pressed in a Buchner press between porcelain or copper plates. The sap obtained in this manner is termed in this paper *vacuolar sap* merely for convenience, since proof is lacking that the liquid obtained is necessarily pure vacuolar sap. The data relating to the extraction of pear leaves with 1.0 N HCl are presented in table I. This table shows that in all samples, with the exception of samples 17 and 18, the amount of iron extracted is higher in the green leaves than in the corresponding chlorotic leaves. This holds true also in the case of samples 13 and 14, in which the total amount of iron present in the green leaves is smaller than that contained in the yellow leaves. Table I thus clearly indicates that a positive correlation exists between the amount of iron extracted from leaves with 1.0 N hydrochloric acid and with their chlorophyll content. It should be emphasized, however, that the two samples (17 and 18) which show exception to this rule were collected late in the season. This fact will be further discussed later.

The data in column 6, however, show also that the total iron in all cases, except samples 13 and 14, is higher in the green leaves than in the corresponding chlorotic ones. This gave rise to the supposition that the amount of iron extracted with 1.0 N hydrochloric acid stood in direct relation to the total iron present in the sample, and that consequently the values presented in column 7 depended on the total iron present, but did not stand in direct correlation to the chlorophyll content of the samples.

In order to test this assumption, a series of pear leaves was collected, and their chlorophyll content, the total amount of iron present, and the amount of iron extracted<sup>3</sup> with 1.0 N hydrochloric acid determined. The

<sup>3</sup> Most of the samples in this series were extracted for 24 hours. While it is not essential to adhere strictly to this period of shaking, it is very important that samples belonging to the same series (e.g., collected from the same trees at a given date) should be extracted for the same length of time.

TABLE I

TOTAL AMOUNT OF IRON IN PEAR LEAVES AND AMOUNT OF IRON EXTRACTED FROM THEM  
WITH 1.0 N HCl

No. OF SAMPLE	CONDI- TION OF LEAVES	DESCRIPTION OF SAMPLE	VARIETY	DATE COLLECTED	TOTAL IRON IN LEAVES	IRON IN 1.0 N HCl EXTRACT
					IN P.P.M. OF DRY WEIGHT OF LEAVES	
1	Green	Spur leaves from one- year-old wood	Bartlett	Apr. 20/29	70	26
2	Chlorotic			"	42	16
3	Green			May 16/29	100	39
4	Chlorotic			"	97	23
5	Green			Aug. 15/29	117	60
6	Chlorotic			"	73	29
7	Green	Leaves from base of shoots	Bartlett	May 29/29	98	47
8	Chlorotic			"	63	27
9	Green	Leaves from middle of shoots	Bartlett	Aug. 17/29	92	47
10	Chlorotic			"	69	32
11	Green	Spur leaves from wood older than one year	Hardy	Apr. 17/29	42	20
12	Chlorotic			"	25	8
13	Green			May 28/29	49	23
14	Chlorotic			"	70	16
15	Green			Aug. 5/29	79	42
16	Chlorotic			"	76	26
17	Green			Aug. 16/27	76	33
18	Chlorotic			"	120	41

sampling was done as follows: severely chlorotic, moderately chlorotic, and green leaves of the same age were collected from the same chlorotic trees. The samples designated as "green, treated with iron in 1928" were obtained from chlorotic trees which were treated with iron in December, 1928, and in consequence of which bore very green leaves in the 1929 and 1930 seasons.

The results of the analyses are presented in table II, columns 6 and 7, from which it is concluded that no correlation exists between the total iron content of these samples and the quantity of iron which is extracted from them with 1.0 N hydrochloric acid. On the other hand, this table reveals

TABLE II  
ACTIVE IRON, CHLOROPHYLL CONTENT, AND AMOUNT OF IRON EXTRACTED FROM HARDY PEAR LEAVES WITH 1.0 N HCl

NO. OF SAMPLE	DESCRIPTION OF LEAVES	LEAVES TAKEN FROM	DATE OF COLLECTING SAMPLE	CHLORO- PHYLL CONTENT % OF DRY WEIGHT OF LEAVES	P.P.M. OF DRY WEIGHT OF LEAVES			
					TOTAL IRON IN 1.0 N HCl EXTRACT	INACTIVE IRON IN 1.0 N HCl EXTRACT	ACTIVE IRON	
1	Severely chlorotic		April 29	0.084	33	14.6	13.2	2.4
2	Moderately chlorotic		"	0.18	40	19.4	13.2	5.1
3	Green, from chlorotic trees		"	0.53	33	27.1	13.2	15.0
4	Severely chlorotic		May 13	0.11	37	9.2	6.4	2.7
5	Moderately chlorotic		"	0.18	27	10.7	6.4	4.5
6	Green, from chlorotic trees		"	0.28	36	12.8	6.4	7.1
7	Green, from trees treated in 1928		"	0.65	51	23.2	6.4	16.2
8	Severely chlorotic		May 27	0.073	32	12.0	10.0	1.2
9	Moderately chlorotic		"	0.15	29	12.0	10.0	2.6
10	Green, from chlorotic trees		"	0.34	32	16.4	10.0	5.9
11	Green, from trees treated in 1928	Spurs	"	0.77	54	22.5	10.0	13.3
12	Severely chlorotic		June 16	0.061	49	14.9	12.3	1.0
13	Moderately chlorotic		"	0.18	47	15.9	12.3	2.9
14	Green, from chlorotic trees		"	0.42	48	17.5	12.3	6.9
15	Green, from trees treated in 1928		"	0.93	63	27.3	12.3	15.2
16	Severely chlorotic		July 9	0.058	62	15.8	14.7	1.4
17	Moderately chlorotic		"	0.18	63	18.9	14.7	3.6
18	Light green from chlorotic trees		"	0.31	64	22.1	14.7	7.2
19	Deep green from chlorotic trees		"	0.44	78	26.1	14.7	10.8
20	Severely chlorotic		"	0.071	76	22.3	19.1	2.0
21	Moderately chlorotic	Base	"	0.22	66	24.0	19.1	6.0
22	Light green from chlorotic trees	of shoots	"	0.29		26.4	19.1	8.0
23	Deep green from chlorotic trees		"	0.68	74	37.4	19.1	18.3
24	Severely chlorotic		"	0.093		21.1	17.7	1.8
25	Moderately chlorotic	Middle	"	0.20	48	19.6	17.7	3.8
26	Deep green from chlorotic trees	of shoots	"	0.65	57	29.9	17.7	12.2
27	Severely chlorotic		"	0.063	59	17.8	16.7	1.0
28	Moderately chlorotic	Terminal	"	0.086	54	17.8	16.7	1.2
29	Deep green from chlorotic trees	of shoots	"	0.60	62	25.0	16.7	8.3

a striking *positive correlation between the amount of iron extracted from the leaves and their chlorophyll content* (see figure 1).

This fact strongly suggests that the active iron, or its hydrolysis product, is contained in the 1.0 N hydrochloric extract. It remains to be determined whether this extract contains *only* the active iron, or whether it contains in addition some other fractions of iron which are inactive.\*

Since iron in these samples is the limiting factor in so far as chlorophyll formation is concerned, it was reasoned that if all the iron in the extract were active iron, then a more or less direct proportionality should exist between the amount of chlorophyll in samples of each series and the total amount of iron extracted from them by 1.0 N hydrochloric acid. On the other hand, if the 1.0 N hydrochloric extract contained also inactive iron, then such a proportionality should exist only between the active iron fraction and the chlorophyll content. The data of table II are plotted in figure 1, where the chlorophyll content is represented by the ordinate, and the total amount of iron extracted from the leaves with 1.0 N hydrochloric acid is plotted on the abscissa. This figure shows that no direct proportion exists between the *total amount* of extracted iron and the chlorophyll content; hence it is concluded that the 1.0 N hydrochloric acid extract contains some inactive iron in addition to the active iron.

It could have been argued that the iron in the 1.0 N hydrochloric acid extract was active iron only, but that this extract contained merely part of the total active iron present in the leaves. If this were true, then sample 14 (table II), for example, which contains about 6.9 times more chlorophyll than sample 12, should contain also about 6.9 times more active iron than sample 12; in other words, it must contain at least  $6.9 \times 14.9 = 102$  p.p.m., which is greatly in excess of the total iron present in sample 15, namely 48 p.p.m.

Figure 1 also indicates that all samples in each series (*e.g.*, samples of the same age, collected from the same trees, and on the same date) lie on a straight line, or their distance from a straight line drawn through them is within the experimental error involved in the determination of their iron content. For most samples in table II the difference between duplicate iron determinations of 1.0 N hydrochloric extracts lies within 5 per cent. of the average value. The error involved in the chlorophyll determination for the values given in this table may therefore amount to as much as 10–15 per cent. This is due in part, presumably, to the variation in chlorophyll content within leaf material of a given sample.

\* The terms *active iron* and *inactive iron* used in this paper refer to the iron fractions which are active or inactive in the formation of chlorophyll only. These terms do not imply anything in regard to the activity of these fractions in other physiological processes, *e.g.*, respiration.

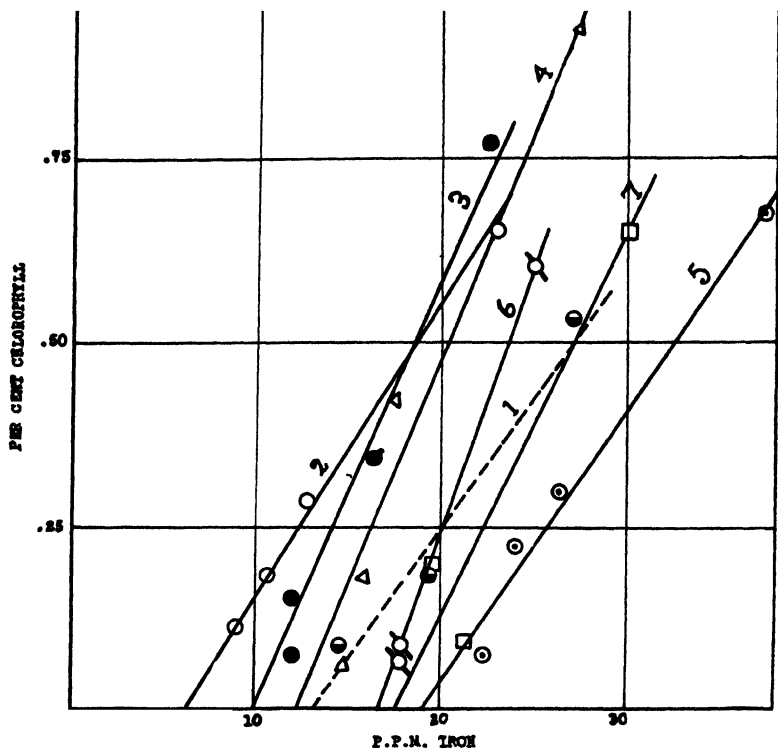


FIG. 1. Chlorophyll content and iron extracted with 1.0 N HCl in 24 hours: 1, ○ = spur leaves collected April 29; 2, ○ = spur leaves collected May 13; 3, ● = spur leaves collected May 27; 4, △ = spur leaves collected June 16; 5, ○ = leaves from base of shoots collected July 8; 6, ○ = leaves from terminal shoots collected July 9; 7, □ = leaves from middle of shoots collected July 7.

This fact may be readily explained on the assumption that *all 1.0 N hydrochloric acid extracts of samples belonging to the same series contain the same amount of inactive iron, and differ only in the amount of active iron they contain.* (The validity of this statement will be discussed later on, and it will be shown that it does not always hold true. This, however, does not affect the conclusions drawn here, since this assumption holds true for the samples given in figures 1 and 5.) Thus the difference in the amount of extracted iron of two samples belonging to the same series corresponds to the difference in the amount of active iron.

If, in figure 1, a straight line be passed through points belonging to samples of one series, then the point of intersection of this line with the abscissa will correspond to a hypothetical sample of chlorotic leaves of which the chlorophyll content is equal to 0. It is evident that the amount of active iron in such a sample must be extremely small, or equal to 0. It

then follows that the distance between this intersection point and the origin represents the amount of the *inactive iron* which a 1.0 N hydrochloric extract of such a sample would contain. But this amount of inactive iron represents, according to our assumption, the amount of inactive iron in the extracts of all other samples of this series; hence the amount of active iron in a sample of this series is given by the expression:  $Fe_a = Fe - Fe_i$ , where  $Fe_a$  is the active iron;  $Fe_i$  is the inactive iron in the 1.0 N HCl extract; and  $Fe$  represents the total iron in the same extract.

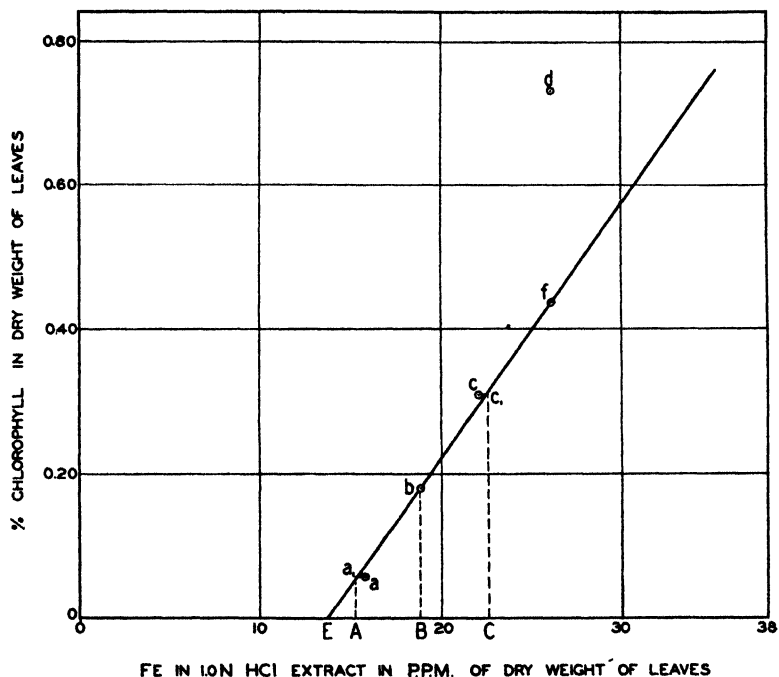


FIG. 2. Estimation of active iron in 1.0 N HCl extract of leaves; Hardy spur leaves collected July 9, 1930.

An example will serve to illustrate the method of estimating the active iron in pear leaves. In figure 2 the points *a*, *b*, *c*, and *f* represent severely chlorotic, moderately chlorotic, slightly green, and very green leaves from chlorotic trees respectively, collected on July 9, 1930. These samples were spur leaves collected from the same trees, and therefore belong to the same series; while sample *d* was collected at the same time and in the same orchard, but from a different group of trees which were treated with iron in 1928. The line *a*, *f* is the straight line of closest fit to pass between the points *a*, *b*, *c*, and *f*. This line intersects the abscissa at the point *E*. Thus *OE* represents the inactive iron for this series of samples. The active iron for

sample  $a$  is obtained by drawing from  $a$  a line parallel to the abscissa; this line intersects  $a_1f$  at  $a_1$ . From  $a_1$  a perpendicular line to the abscissa is drawn which intersects it at  $A$ ;  $EA$  is thus the active iron for  $a$ , while  $EB$  and  $EC$  represent the active iron for  $b$  and  $c$  respectively. The values of Fe for several series of Hardy pear leaves are plotted in figure 1 against the chlorophyll content, and straight lines are drawn through points belonging to the same series, which allow the estimation of the active iron. The values thus obtained are presented in table II, column 9.

In figure 3 the values of  $Fe_a$  are plotted on the ordinate against the value of  $(Fe - Fe_l)$  on the abscissa on the same scale. The points on figure 3 represent 29 samples comprising 8 series and collected at different times of the season between April and July. These samples include spur leaves as well as shoot leaves,<sup>5</sup> all of which were collected from Hardy trees in a chlorotic orchard. If  $Fe_a$  were equal to  $(Fe - Fe_l)$ , then all the points

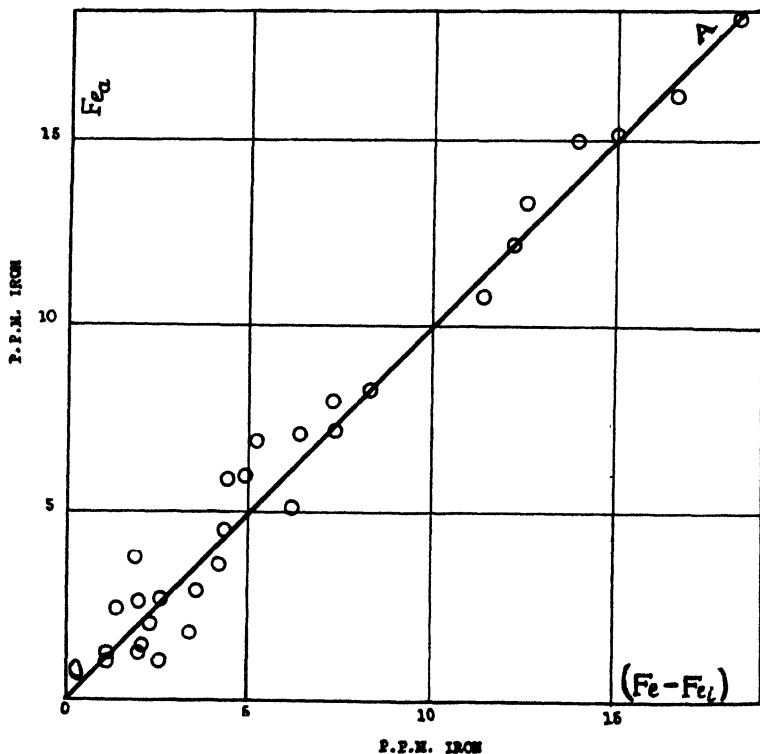


FIG. 3. Active iron ( $Fe_a$ ) and values of  $(Fe - Fe_l)$  for Hardy pear leaves.

<sup>5</sup> In the case of shoot leaves, each series comprised leaves of nearly the same age. This was accomplished by segregating the leaves from the base, middle, and top of the shoots to make separate samples.

in figure 3 should lie on a straight line,  $OA$ , passing through the origin and making an angle of  $45^\circ$  with either one of the axes. The positions of the points on figure 3 afford a means of gauging the agreement between the calculated values of the active iron (that is,  $Fe_a$ ) and the observed values. This figure shows that out of 29 samples there is not a single one for which the value of  $\Delta Fe$ , as defined by the expression  $\Delta Fe = Fe_a - (Fe - Fe_t)$ , equals or exceeds 2 p.p.m., and for 22 samples  $\Delta Fe$  is less than 1 p.p.m. This is very good agreement, since the values of  $\Delta Fe$  are well within the limit of the error involved in the determination of  $Fe$ .

The data plotted in figures 1 and 2 pertain only to the samples which were collected up to July 9; samples collected later do not show a consistent correlation between the amount of iron extracted in 24 hours with 1.0 N HCl and their chlorophyll content, as can be seen from table III and from figure 4 which represents the results obtained for Hardy samples collected on August 7. This finding is in agreement with the data reported in table I (samples 17 and 18), in which it was shown that the 1.0 N HCl

TABLE III

CHLOROPHYLL CONTENT, AND THE IRON EXTRACTED WITH 1.0 N HCl FROM PEAR LEAVES COLLECTED FROM CHLOROTIC TREES LATE IN GROWING SEASON

DESCRIPTION OF LEAVES	VARIETY	ORCHARD	DATE OF COLLECTING SAMPLES	CHLOROPHYLL CONTENT IN % OF DRY WEIGHT OF LEAVES	TOTAL IRON EXTRACTED WITH 1.0 N HCl IN P.P.M. OF DRY WEIGHT OF LEAVES
Severely chlorotic	Bartlett	Mc	July 20	0.087	53.3
Moderately chlorotic	"	"	"	0.27	43.3
Light green	"	"	"	0.42	39.5
Deep green	"	"	"	0.68	54.1
Severely chlorotic	Hardy	M	"	0.10	16.4
Moderately chlorotic	"	"	"	0.26	25.0
Light green	"	"	"	0.43	22.5
Deep green	"	"	"	0.96	36.1
Severely chlorotic	"	"	August 7	0.056	28.1
Moderately chlorotic	"	"	"	0.20	22.0
Light green	"	"	"	0.25	30.0
Deep green	"	"	"	0.66	34.2
Severely chlorotic	"	B	"	0.045	43.1
Moderately chlorotic	"	"	"	0.14	38.5
Light green	"	"	"	0.38	40.4
Deep green	"	"	"	0.73	54.8

extract of a chlorotic sample collected in August contains more iron than a similar extract from green leaves.

An explanation for this fact may be furnished by the observations of SACHS (10) and other workers. SACHS noticed that chlorotic leaves did not respond to treatment of iron when applied late in the season. ZIMMERMAN (17) and ROUX (9) reported that the chloroplasts of severely chlorotic leaves showed marked signs of injury, which finally resulted in the disintegration of the plastids. These observations indicate that some profound changes take place in chlorotic leaves late in the season, which may affect the solubility of the various iron compounds of the leaves. It is likely that the hydrochloric acid extract of chlorotic samples collected late in the season may contain amounts of inactive iron different from those of the extracts from green leaves. The active iron cannot be determined in samples collected late in the season, since the method of its estimation, previously described, can be used only when all the samples of a series contain in their hydrochloric acid extract the same amount of inactive iron.

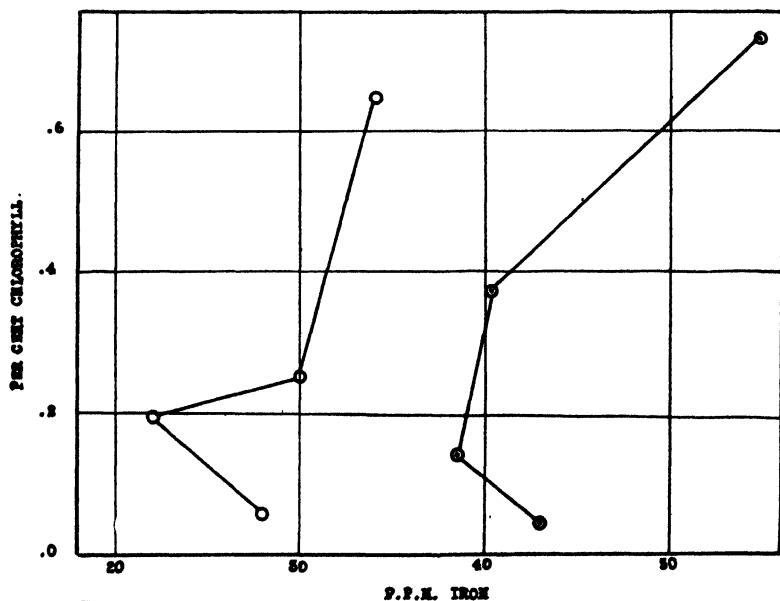


FIG. 4. Iron extracted from Hardy spur leaves with 1.0 N HCl; samples collected August 7: —○—, leaves from orchard M; —●—, leaves from orchard B.

Again, the injury to the leaf caused by prolonged chlorosis may impair the efficiency of the active iron, until the chloroplasts are injured beyond recovery, when the active iron, even if abundant, may fail to bring about the formation of chlorophyll. If this were the case, then the amount of

active iron in leaves of the same age, collected from the same trees, would not be proportional to their chlorophyll content.

From the foregoing it is obvious that the method of determining the active iron is limited in its application for the following reasons:

1. In order to estimate the active iron in any one sample, it is necessary to determine the chlorophyll content and the acid-extractable iron of a whole *series* of samples (the series should consist of at least three samples).

2. The leaf samples in each series must be of the same age and grown on the same trees; they must, however, differ markedly in their chlorophyll content.

3. The active iron must be the limiting factor in each sample of a series, in so far as chlorophyll formation is concerned.

4. The method cannot be applied to samples collected late in the season (*e.g.*, August or later).

On account of these limitations, a method which could be of wider application is being investigated at the present time.

#### IV. Active iron in peach and apricot leaves

Green and chlorotic peach and apricot leaves were collected from chlorotic trees. The chlorophyll content, total amount of iron, and iron extracted from the leaves with 1.0 N HCl were determined by the same procedure as used with pear leaves. The data obtained are presented in table IV and figure 5. It may be noted that while the total amount of iron in the chlorotic leaves is smaller than that present in the green leaves,

TABLE IV

ACTIVE IRON IN PEACH AND APRICOT LEAVES FROM CHLOROTIC TREES. SAMPLES COLLECTED JULY 20 FROM MIDDLE OF SHOOTS

DESCRIPTION OF LEAVES	CHLOROPHYLL CONTENT IN % OF DRY WEIGHT	TOTAL IRON IN LEAVES	IRON IN 1.0 N HCl EXTRACT	ACTIVE IRON
		IN P.P.M. OF DRY WEIGHT OF LEAVES		
Severely chlorotic peach leaves . . .	0.21	41	14.0	3.8
Moderately chlorotic peach leaves ..	0.68	48	22.3	10.1
Light green peach leaves . . . . .	1.09	75	30.2	19.6
Deep green peach leaves . . . . .	1.47	75	36.9	26.7
Severely chlorotic apricot leaves . . .	0.16	48	15.5	5.1
Moderately chlorotic apricot leaves	0.31	45	18.0	9.4
Light green apricot leaves . . . . .	0.59	76	30.8	17.8
Deep green apricot leaves . . . . .	0.90	62	37.7	27.2

no consistent relation exists between the total iron content and the chlorophyll content, while the relation between the amount of iron extracted with 1.0 N HCl and the chlorophyll content is very close. It is evident that the method of estimation of active iron as described in this paper is applicable to leaves of pear, apricot, and peach, and presumably also to other material.

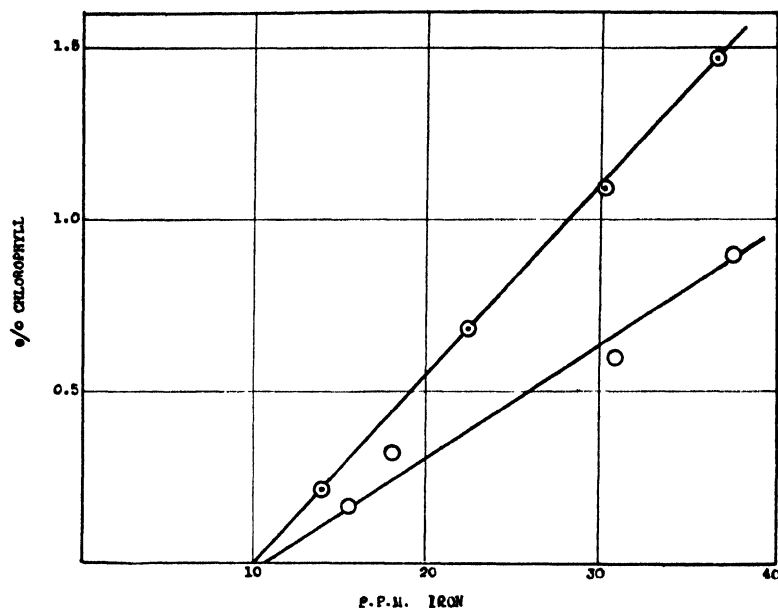


FIG. 5. Chlorophyll content and iron extracted from 1.0 N HCl from peach leaves ( $\odot$ ) and apricot leaves ( $\circ$ ).

### V. Nature of active and inactive iron

When a solution of ammonium thiocyanate or potassium thiocyanate is added to 1.0 N HCl extract of pear leaves, the brown liquid turns red-brown. This indicates the presence of  $\text{Fe}^{+++}$  ions, or of an iron compound readily converted into  $\text{Fe}^{+++}$ . The 1.0 N HCl extract is deeply colored, however, owing to the presence of decomposition products of chlorophyll and of other compounds. For this reason the intensity of the red color of the iron thiocyanate cannot be determined directly on the extract. In order to separate the ionic iron from the rest of the extract, the following procedure was adopted.

The 1.0 N HCl extract of a leaf sample was made up to 175 cc. with 1.0 N HCl solution, and 25 cc. of 40 per cent.  $\text{NH}_4\text{CNS}$  were added to it. To this solution 50 cc. of ethyl acetate were added; the whole was shaken for a minute or two in a separatory funnel, the emulsion allowed to stand for 10–20 minutes, the aqueous phase drained, and the ethyl acetate collected

in a flask. The extraction with ethyl acetate was repeated five or six times until the ethyl acetate remained colorless. The ethyl acetate extracts were added together and evaporated slowly in pyrex beakers on a hot plate at a low heat. When the volumes of the liquid in the beakers were reduced to about 10–20 cc., the beakers were removed from the hot plates and allowed to cool, and concentrated nitric acid was added drop by drop, a few drops at a time with an interval of several minutes between each addition. After this process was repeated several times, about 5 cc. of concentrated nitric acid were added and the liquid in the beakers was evaporated at low heat until dry. The beakers were then put in an electric furnace and the residue ashed at low temperature (at a very dull red). The determination of the iron in the ash was carried on in the manner previously described.

TABLE V

IONIC AND IONIZABLE IRON IN 1.0 N HCl EXTRACTED FROM PEAR LEAVES

DESCRIPTION OF SAMPLE	DATE OF COLLECT- ING SAMPLE	HOURS OF EXTRAC- TION WITH 1.0 N HCl	TOTAL IRON IN 1.0 N HCl EXTRACT	IRON IN ETHYL ACETATE EXTRACT	IRON IN ACID RESIDUE	Fe <sub>a</sub>
			IN P.P.M. OF DRY WEIGHT			
Severely chlorotic spur leaves	July 9	44	12.8	8.3	4.5	1.4
Light green spur leaves . . . . .	“	47	24.7	21.2	3.5	7.2
Light green leaves from shoot ter- minals . . . . .	“	7	15.2	12.7	2.5	8.3

The results of the ethyl acetate extractions are presented in table V. Practically all the iron in the HCl extract was removed by ethyl acetate. The small quantities found in the acid residue may have been due partly to traces of iron in the reagents used, and partly to some ethyl acetate which remained as a fine emulsion in the acid phase. The data in table V indicate that *practically all of the iron in the acid extract is present as ferric iron, or in a form which is readily converted into Fe<sup>+++</sup>*. In this respect no difference exists between the active and the inactive iron in 1.0 N HCl.

This fact, however, does not disclose in what form the active and the inactive iron respectively are present in the living cell. So much, however, can be concluded: *these two forms of iron are present in the leaf cells in compounds which can be dissolved, or readily converted by 1.0 N HCl into ionic iron or ionizable iron*. It may be of interest to note that all the iron

compounds used successfully in this investigation for the cure of chlorosis were compounds which in aqueous solutions dissociate, at least partly, into ferric or ferrous ions. An attempt to treat chlorotic trees with potassium ferrocyanide, which in aqueous solution does not yield ionic iron in appreciable amounts, was not successful on account of the injurious effects of potassium ferrocyanide on the trees. No conclusion, therefore, can be drawn from this experiment regarding the ability of pear leaves to convert a non-ionic iron compound into active iron.

Since it has been found in several leaf samples that the amount of active iron greatly exceeds the amount of iron extracted with water, or the amount of iron contained in the "vacuolar sap," it is thus inferred that *the active iron is not present in these leaf samples in solution; at the most only part of it is soluble.*

Pear leaf tissue tested microchemically for iron with potassium ferrocyanide and potassium ferricyanide yielded negative results. Positive tests were obtained only with leaves which were taken from trees treated with iron. The positive reaction in these leaves was observed only in and near parts which showed injury effects due to an excess of iron. The microchemical tests thus carried out were not numerous, but the results obtained are in agreement with those of MILAD (8). This is not necessarily proof that no ionic iron readily soluble in hydrochloric acid exists in pear leaves. It has already been shown that the amount of active iron in pear leaves is not large, and it probably seldom exceeds 50-80 p.p.m. of the dry weight; often it is much less than that. Such an amount of iron when distributed in an excess of reagent may be diluted to the extent that it remains in solution notwithstanding the presence of large amounts of ferri- or ferrocyanide.

TABLE VI

VALUES OF E FOR VARIOUS LEAF SAMPLES

LEAVES COLLECTED FROM	PLANT	DATE OF COLLECTING SAMPLE	E
Spurs . . . . .	Hardy pear	April 29	22.1
Spurs . . . . .	"	May 13	25.1
Spurs . . . . .	"	May 27	37.7
Spurs . . . . .	"	June 16	38.3
Spurs . . . . .	"	July 9	22.0
Base of shoots . . . . .	"	July 9	22.7
Middle of shoots . . . . .	"	July 9	33.2
Terminal end of shoots . . . . .	"	July 9	44.3
Middle of shoots . . . . .	Peach	July 20	33.4
Middle of shoots . . . . .	Apricot	July 20	20.7

It is also possible that an appreciable fraction of the ionic iron is present as complex in the HCl extract, tied up with organic acids. The iron in these complexes may be readily ionizable, but the concentration of  $\text{F}^{++}$  or  $\text{Fe}^{+++}$  in the extract may be too small to yield a positive test with  $\text{K}_3\text{Fe}(\text{CN})_6$  or  $\text{K}_4\text{Fe}(\text{CN})_6$ .

The slope of the lines in figure 1 and figure 5 is given by the ratio  $E = \frac{\text{chlorophyll content}}{\text{active iron}}$  per unit weight of leaves. This ratio is therefore a measure of the efficiency of the active iron in chlorophyll formation. In table VI the values of  $E$  are presented for ten series of samples. The values of  $E$  in this table are expressed in mols of chlorophyll<sup>6</sup> per gram atom of active iron. No consistent correlation is revealed between the age of the leaves and the value of  $E$ . The fact that the values of  $E$  are large and variable leads to the conclusion that it is *highly improbable that the active iron is combined with the chlorophyll in a stoichiometrical relation* if it is combined with it at all.

Iron is known to catalyze oxidation processes in living substances. The formation of chlorophyll in the living plants is, most likely, associated with an oxidation process, since several investigators claimed that the rate of chlorophyll formation in seedlings was greatly impeded by low oxygen pressure in the air surrounding the plants (for example, CORRENS 4). The active iron presumably does not form a part of the chlorophyll molecule; it is likely, therefore, that its function consists in catalyzing an oxidation process or some oxidation processes connected with chlorophyll formation.

EMERSON (5) succeeded in growing *Chlorella* in sugar solutions deficient in iron. The algae in such solutions were devoid of, or deficient in, chlorophyll; but their rate of respiration was substantially the same as that of normally green *Chlorella*. The fact that the chlorotic algae had a normal respiratory rate suggests that they were not subnormal in regard to the amount of "respiratory ferment" they contained. In spite of this they were decidedly subnormal in their chlorophyll content. It seems, therefore, improbable that the active iron is identical with the respiratory iron ferment of WARBURG (15).

The nature and the localization in the cell of the inactive iron are also uncertain. The inactive iron extracted with 1.0 N HCl may differ from the active iron only in regard to its localization in the cell, *i.e.*, it may be present only in the interior of plastids or other protoplasmic bodies. On the other hand, it is equally plausible that the inactive iron compound may differ from the active in its chemical composition; nor is it certain that the

<sup>6</sup> One mol of chlorophyll was taken as equal to 897.4 which represents an average value for chlorophyll ( $a + b$ ), on the assumption that the ratio of chlorophyll  $a$  to chlorophyll  $b$  is 3.

inactive iron in the acid extract is derived from one compound only. This last statement may be applied also to the active iron.

The writer wishes to emphasize that while it is common to find chlorotic leaves which contain as much or more iron than green leaves of the same age, the inability to utilize iron for a normal development of chlorophyll is confined to leaves with a low content of iron during at least the first part of the growing season. This statement does not necessarily apply, of course, to yellow leaves in which the development of chlorophyll is abnormal, due to other causes than those which are responsible for lime-induced chlorosis.

This fact would indicate that the occurrence of chlorosis is not entirely independent of the amount of iron in the leaves. Indeed, a comparison of the iron content of leaves in the chlorotic orchard C with that of green leaves from orchard S, in a region free of chlorosis (fig. 6), shows that the

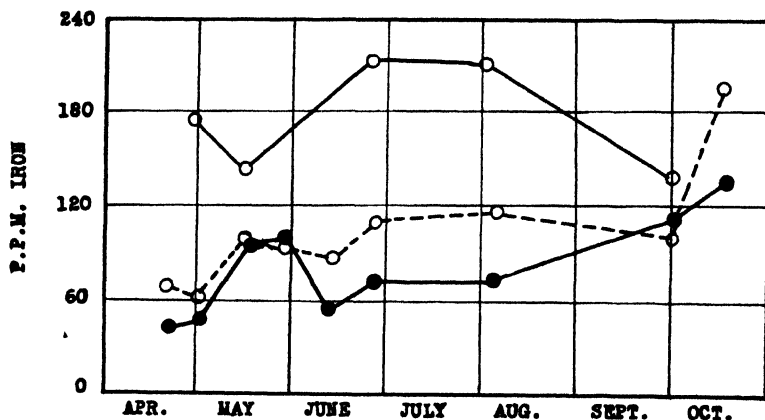


FIG. 6. Seasonal variation of iron content (on dry-weight basis) in Bartlett spur leaves of one-year-old wood: —●—, chlorotic leaves, orchard C; ---○---, green leaves, orchard C; —○—, green leaves, orchard S.

iron in leaves from orchard S is on a higher level *throughout* the period of active growth. From experience the writer is inclined to conclude that the occurrence of chlorosis is highly improbable in pear leaves, the iron content of which remains above, say, 70–80 p.p.m. (on the dry-weight basis) during the first two or three months of their growth. An explanation for this fact is offered by the suggestion that a certain equilibrium exists between the active iron and the inactive iron in pear leaves. In leaves containing a small amount of iron, the balance between the two forms of iron may be shifted in such a way as to prevent the formation of an adequate amount of active iron for normal chlorophyll formation; while in leaves rich in iron, the active iron (although it may be only a small fraction of the total

iron) is present in sufficient amount for the normal development of chlorophyll.

### Summary

1. Chlorotic pear leaves may contain as much or more iron than green leaves of the same age and taken from the same trees, regardless of whether the iron content is expressed on the fresh-weight or the dry-weight basis. The iron content, however, of green leaves from trees grown in districts free from chlorosis is higher than the iron content of either green or yellow leaves from chlorotic trees. Lime-induced chlorosis (dealt with in this paper) is confined to leaves in which the iron content is relatively low during the first two or three months of the growing season.

2. No correlation exists between the amount of iron extracted from pear leaves with water and with 0.5 N HCl and the chlorophyll content of leaves.

3. Only part of the iron in leaves, the *active iron*, is effective in chlorophyll formation.

4. A method is described for the estimation of the active iron in leaves, which is based on the assumption that the active iron, or its derivative, is contained in the 1.0 N HCl extract of dried leaves.

5. The chlorophyll content of leaves from chlorotic plants is proportional to the amount of active iron in the leaves.

6. The iron of the compound active in chlorophyll formation is present in the 1.0 N HCl extract as ionic iron or in a compound which readily yields ionic iron.

7. The values of the ratio  $\frac{\text{chlorophyll}}{\text{active iron}}$  in leaves varies widely in different sets of leaves. It is inferred from this fact that the active iron is not present in leaves in a stoichiometrical combination with chlorophyll, if it is combined with it at all.

The writer wishes to express his appreciation to Dr. J. P. BENNETT for the valuable assistance, suggestions, and criticisms offered throughout the work.

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# INDIRECT COLORIMETRIC METHOD FOR THE DETERMINATION OF CALCIUM<sup>1</sup>

E. M. EMMERT

## Introduction

The method described in this paper enables the rapid and accurate determination of calcium in a series of samples which do not differ greatly in their calcium content. As with other colorimetric methods, it is particularly useful when small amounts of the element are to be determined. It differs from other colorimetric methods in that large amounts of Ca can also be determined accurately by proper adjustment of reagents.

When the amount of calcium is relatively large and large differences from sample to sample are to be expected, the oxalate titrimetric method of McCrudden (5) would be preferred, since under these conditions numerous preliminary tests would be necessary in the colorimetric method here presented. This colorimetric method, however, is especially well adapted to detect the effect of varying treatments on the calcium content of biological material in which these treatments cause only small but significant variations in the calcium content. In this case few preliminary tests would be necessary. If phosphorus is to be determined at the same time as calcium, the colorimetric method enhances rapidity as well as increases accuracy for small amounts of calcium.

## Basic principles

When excess sodium hydroxide is added to a solution containing iron, magnesium, calcium, and phosphate, in the absence of ammonia salts, iron and magnesium are precipitated as hydroxides while calcium is precipitated as tricalcium phosphate. Iron and magnesium phosphates are not precipitated because they are more soluble than the hydroxides of these metals. Calcium hydroxide, however, is relatively soluble as compared with tricalcium phosphate; therefore only calcium causes a reduction in the phosphate content of the alkaline solution. This reduction of phosphate may be measured very accurately by the colorimetric method for phosphorus, and from this the amount of calcium may be calculated.

## Interference

Significant amounts of the ammonium ion would interfere since magnesium hydroxide would tend to be dissolved and magnesium ammonium

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published with permission of the Director.

phosphate precipitated. The ammonium ion may be eliminated in the usual ways, such as by igniting the salts or boiling with strong sodium hydroxide.

Any material which colors the solution and is not precipitated out of the solution by sodium hydroxide will interfere. If the color is organic it may be oxidized and eliminated by sodium chlorate (1) or ignition in a muffle furnace. Aluminum and most other metals likely will act similarly to iron and not interfere, although if some such elements are present in significant amounts their effect on phosphate in alkaline solution should be tested.

If the amount of phosphate in the sample is large as compared with the calcium present, the method is not very accurate, since the reduction in color by the calcium will be too small. This may be overcome by adding a known amount of calcium to reduce the phosphate color to the correct point and computing the calcium by subtracting the added calcium from the total calcium found.

### Determination of phosphate

The exact amount of phosphate in the unknown must be found preliminary to the calcium determination. Take a suitable neutral aliquot (10–70 cc.), make to about 70 cc., add 10 cc. of 2.5 per cent. ammonium molybdate in 5 N sulphuric acid and 3 cc. of 1, 2, 4 amino-naphthylsulphonic acid (4), and make up to 100 cc. After five minutes compare with a standard blue phosphate solution which was prepared at the same time as the unknown. This is made by developing the color from 1 cc. of the dilute phosphate standard (reagent 3) as just described.

### Approximate determination of calcium

To a 20-cc. aliquot of the calcium unknown solution add enough phosphate to make about 0.3 mg. of P with the phosphate already in the unknown. Make alkaline to phenolphthalein with 10 per cent. sodium hydroxide and add 2 cc. in excess. Make up to 50 cc., shake, and filter through a dry filter. Test a 20-cc. aliquot for phosphate. If no blue color appears, more than 0.6 mg. of calcium is present. To another 20 cc. of the filtrate add enough phosphate standard to make about 3 mg. of P. Shake intermittently for a few minutes and filter. If no blue color appears, more than 6 mg. of calcium are present. Repeat with varying amounts of phosphate until a rough approximation of the calcium is obtained, judging the amounts from the colors developed.

### Accurate determination of calcium

The exact quantity of phosphate in the unknown solution and the approximate range of calcium must have been determined as directed previously. For a series of samples of nearly the same calcium content one approximate determination of calcium is sufficient.

To an appropriate aliquot of the calcium unknown add sufficient standard phosphate so that, with what is already in the solution, the total amount is brought up to about the amount indicated in table I for the approximate range of calcium as already found in the sample. The exact phosphate present must be known and should be sufficient so that the calcium present will precipitate between one-fourth and three-fourths of the phosphate present. Add a few drops of phenolphthalein and calcium-free 10 per cent. sodium hydroxide solution until a pink color persists. Add 2 cc. of the sodium hydroxide in excess, shake intermittently for about a minute, and make to 50 cc. Shake a few seconds and filter through a dry no. 2 Whatman filter or through any filter as retentive as this type of paper. Take an aliquot such that the phosphate present is in the range of about 0.1 to 1.0 mg. of phosphate phosphorus. Add enough dilute sulphuric acid just to dispel the pink phenolphthalein color. Determine the phosphate phosphorus left as directed for determining the phosphate in the sample. From the quantity of phosphate in the solution before making it alkaline, and that left after making it alkaline and filtering, the exact amount of calcium may be calculated.

TABLE I

AMOUNTS OF PHOSPHATE STANDARD TO BE USED

APPROXIMATE MG. OF CA	AMOUNT OF P NEEDED	P REAGENT	CA MILLIGRAM EQUIVA- LENTS OF P SOLUTION
<i>mg.</i>	<i>mg.</i>	<i>cc.</i>	<i>mg.</i>
0.1- 0.5 . . . . .	0.3099	1 (dilute)	0.6
0.5- 1.0 . . . . .	0.6198	2    "	1.2
1.0- 5.0 . . . . .	3.0986	10   "	6.0
5.0-10.0 . . . . .	6.1972	20   "	12.0
10.0-20.0 . . . . .	12.3944	3 (concentrated)	24.0
20.0-30.0 . . . . .	18.5916	6    "	36.0
30.0-40.0 . . . . .	24.7888	8    "	48.0

## Reagents

1. Concentrated standard phosphate solution: dissolve 3.398 gm.  $\text{KH}_2\text{PO}_4$  in distilled water and make to 250 cc.; 1 cc. equals 3.0966 mg. of P, which is equivalent to 6 mg. of calcium.

2. Dilute standard phosphate solution: dilute 10 cc. of reagent 1 to 100 cc.

3. Standard calcium solution: dissolve 0.2498 gm.  $\text{CaCO}_3$  in distilled water and just enough  $\text{HCl}$ , and make to 250 cc.; 1 cc. equals 0.4 mg. of Ca.

## Results

Table II shows the results of calcium determinations made in the presence of iron and magnesium.

TABLE II  
DETERMINATION OF CALCIUM IN PRESENCE OF IRON AND MAGNESIUM

FE PRESENT	Mg PRESENT	CA ADDED	CA FOUND	PERCENTAGE ERROR
<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>%</i>
1.0	0.4	0.1	0.1	0.0
0.4	0.4	0.4	0.400	0.0
0.4	0.4	0.4	0.399	- 0.25
0.4	0.4	0.4	0.397	- 0.75
0.4	0.2	1.6	1.590	- 0.60
4.0	2.0	2.0	2.050	2.50
4.0	2.0	2.0	2.050	2.50
8.0	4.0	2.0	2.003	0.15
4.0	2.0	4.0	4.000	0.0
8.0	4.0	4.0	3.981	- 0.47
8.0	4.0	4.0	4.098	2.45
8.0	4.0	4.0	3.981	- 0.47
8.0	4.0	8.0	7.930	- 0.88
4.0	4.0	20.0	20.3	1.5
4.0	4.0	20.0	20.0	0.0
4.0	4.0	0.0	0.0	0.0
8.0	4.0	0.0	0.0	0.0

## Discussion

ROE and KAHN (6) use the same principle in their colorimetric method, but work with the precipitated phosphate instead of the excess phosphate. Their paper is the only one found which was at all similar to the method of the present paper, and their results simply give further proof of the accuracy of the method used in this paper.

The main objection to the method of ROE and KAHN is that centrifuging and special technique are required in washing out the excess phosphate, which increases the labor and endangers accuracy. Incomplete washing or a slight dissolving of the phosphate is likely to cause error.

Special care must be taken to wash out excess phosphate if colloidal hydroxes of iron, manganese, and aluminum are present, since they would tend to retain the phosphate by adsorption. They will also dissolve along with the phosphate and interfere in the colorimetric phosphate test. Other interfering substances precipitated by NaOH would also be dissolved.

A large excess of phosphate does not seem necessary so long as a sufficient excess of OH<sup>-</sup> ions is present. Of course, the OH<sup>-</sup> ions should not be too

concentrated, since there is danger of redissolving the calcium phosphate; but this is not possible in the procedure here presented, since pH 13 or more is necessary according to ROE and KAHN, and the concentration of the NaOH here used is only slightly more than 0.1 N. No phosphate test could be secured in the filtrate when a solution containing 0.2 mg. of phosphorus was treated with 0.8 mg. of calcium and 0.1 normal NaOH and filtered. This proves that the complete precipitation of calcium phosphate does not depend on the common ion effect in the presence of OH<sup>-</sup> ions, and only a small excess of phosphate as used in the present method would insure accurate results.

The principle of reducing color has been used previously for determining potassium (3) and carbon dioxide (2). This principle deserves as much recognition in colorimetry as titration of excess acid and alkali does in volumetric analysis. As yet its use is very limited.

The mathematical progression involved makes the colorimetric readings as the color is progressively reduced increasingly accurate. As the calcium takes out the phosphate the readings become very accurate. It is apparent that by adjusting the phosphate properly, colorimetric readings more accurate than with the ordinary increase in color intensity can be secured, since such readings approach infinity as the color loses intensity while the ordinary colorimetric readings approach zero with lessened color intensity.

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## BRIEF PAPERS

### POSSIBLE CHANGES IN THE WAXLIKE COATING OF APPLES CAUSED BY CERTAIN SPRAY AND OTHER TREATMENTS<sup>1</sup>

A number of investigators have studied the effects of arsenic, sulphur, and copper sprays on the chemical composition of fruits, particularly citrus fruits and apples. With citrus fruits (1, 3, 4, 5, 8, 11, 12) it has been definitely established that the use of arsenical sprays causes certain changes in acid, sugar, and vitamin C, while in the case of apples (2) a possible effect of certain fungicides upon the chemical composition has been indicated. Furthermore, results obtained by other workers (6) who have tried to determine the direct effects of the use of oil seem to indicate that its application affects the physiological behavior of the plant.

In view of the wide-spread interest and importance attached to these studies, it seemed desirable to report data dealing with possible changes in the waxlike coating of apples caused by certain spray and other treatments. While it is difficult, at the present time, definitely to evaluate the effect of changes in the condition of the cuticle on the general metabolism within the fruit, it appears very probable that skin condition may play an important rôle through its indirect effect on respiratory activity.

Besides being of interest in connection with the physiological behavior of the plant, the study of the effect of different spray treatments on the apple is of particular significance in connection with the spray-residue removal problem. In the west a large percentage of the harvested apple crop is washed with chemicals to reduce the quantity of arsenic remaining from spray materials to the amount permitted by law, and it is claimed that the successful removal of the residue is often prevented or rendered difficult by the use of oil as a component of the spray mixture used for the control of codling moth. One of the explanations offered (10) to account for this difficulty is based on the assumption that the oil from the spray mixture coats the lead arsenate particles and protects them from the solvent action of the acid employed to remove the arsenic. While it is known that mineral oil penetrates leaves by way of the stomata (6), it is not definitely known whether the oil sprayed on apples remains on the surface admixed with the natural waxlike coating or volatilizes before harvesting time, as claimed by REGAN (9). In one of the apple cleaning processes used, the fruit is first treated with an alkaline wash at about 110° F., then washed, brush-dried, and atomized with a slightly warmed

<sup>1</sup> From the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, in cooperation with the Food Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

mixture of mineral oil and paraffin wax. Examination of the cuticle of apples thus treated should indicate the effect of such treatment on the quantities of natural waxlike constituents.

Through the kindness of F. L. OVERLEY and A. SPULER, of the Washington Agricultural Experiment Station, it was possible to examine Jonathan apples from Wenatchee, Washington, some of which were unsprayed and others of which were subjected to different spray treatments. All of the trees from which these samples were collected were located rather close to one another in the same orchard. Fruit was sampled within a few hours after harvesting. Two samples of Jonathan apples grown in Yakima, Washington, were also examined, one before, and the other after, receiving a treatment consisting of an alkaline wash followed by atomizing treatment with mineral oil and paraffin wax.

The results of this study are presented in table I. The methods used in sampling and in determining the apple surface constituents, namely, ursolic acid, oily fraction, and total ether extract, are the same as those previously reported in detail (7). The method of determining cutin will be reported in a later paper. Briefly, it consisted of hot acid and alkali extraction of the cuticle, after extraction with ether and alcohol, and saponification of the remaining cutin with alcoholic potash, the loss in weight after saponification being considered the amount of cutin.

It will be seen from an examination of the data that individual as well as averaged values for ursolic acid, oily fraction, and total ether extract representing the non-oil-sprayed and the glycerin and oil-sprayed fruit are higher than corresponding values representing the check or unsprayed fruit. With but few exceptions the individual values are higher for the oil-sprayed than for the non-oil-sprayed samples. In those cases where cutin was determined the oil-sprayed samples show higher values than the non-oil-sprayed samples and the unsprayed fruit. With fruit treated with an alkaline wash and then atomized with a mixture of mineral oil and paraffin wax, the oily fraction appears to be increased by the applications.

The data indicate that the ursolic acid and oily fraction increase in approximately the same proportions; therefore it is not believed that any appreciable increase in the naturally occurring oily fraction can be ascribed to a direct accumulation on the surface of the apple of mineral oil from the spray mixture, provided the mineral oil is of relatively low viscosity and the oil spray is not applied too late in the season. It seems, therefore, that the difficulty experienced in removing spray residues by cleaning processes cannot be attributed directly to an accumulation of mineral oil from the applied spray, but rather to an increase of all the ether-soluble waxlike constituents resulting from a physiological stimulation. It should be borne in mind, however, that the application of mineral oil to the surface of the fruit might

TABLE 1  
EFFECT OF SPRAY AND OTHER TREATMENTS ON THE SURFACE CONSTITUENTS OF JONATHAN APPLES

SOURCE AND TREATMENT	CONSTITUENTS PER 100,000 SQUARE MILLIMETERS OF SURFACE AREA				OILY OR PE- TROLEUM-ETHER SOLUBLE FRACTION IN TOTAL ETHER EXTRACT
	URSOLIC ACID	OILY OR PE- TROLEUM-ETHER SOLUBLE FRACTION	TOTAL ETHER EXTRACT	CUTIN	
	mg.	mg.	mg.	mg.	%
WENATCHEE, WASHINGTON:					
Unsprayed	481.0	321.5	802.5	946.4	40.0
Lead arsenate* and spreader,† calyx and covers 1-6	531.6	361.2	892.8		40.5
Lead arsenate* and spreader,† calyx and covers 1-6	537.4	369.4	906.8		40.7
Lead arsenate,* calyx and covers 2-6; lead arsenate,* pyrethrum (1-800), cover 1	567.4	334.4	901.8	969.5	37.1
Lead arsenate,* calyx and covers 1-5	516.0	339.8	855.8	888.6	39.7
Mean for non-oil-sprayed fruit	538.1	351.2	889.3	929.0	39.5
Lead arsenate,* calyx and covers 1, 2, 3, 4; oil† (70-75) and nicotine sulphate (1-1600), covers 5, 6	565.7	398.7	964.4	1080.2	41.3
Lead arsenate,* calyx and covers 1, 3, 4, 5; oil† (70-75) and nicotine sulphate (1-1600), covers 2, 6	559.2	384.2	943.4	1048.4	40.7
Lead arsenate,* calyx and covers 2, 3, 4; glycerin (1 qt.- 100) and nicotine sulphate (1-800), covers 1, 5, 6	580.0	365.5	945.5		38.7
Lead arsenate,* calyx and covers 1-5; lead arsenate,* oil† (70-75) and spreader (1/2-100), cover 6	573.2	360.1	933.3	1030.9	38.6
Lead arsenate* and spreader,† covers 1-5; lead arse- nate,* oil† (70-75) and spreader, cover 6	574.2	386.2	960.7		40.2
Lead arsenate,* calyx and covers 1-5; lead arsenate,* oil† (50) and spreader,† cover 6	561.6	398.5	960.1		41.5
Lead arsenate,* oil† (50), calyx and covers 1-6	564.8	377.2	942.0	1082.6	40.0
Mean for glycerin and oil-sprayed fruit	568.4	381.5	949.9	1060.5	40.2
YAKIMA, WASHINGTON:					
Lead arsenate,* calyx and covers 1, 2; lead arsenate* and oil (1 per cent.) cover 3 about July 10	555.1	352.0	907.1	1094.7	38.8
Fruit similar to above, after alkaline wash and atomiz- ing treatment with mineral oil and paraffin wax	554.4	410.6	965.0	1117.8	42.5

\* Two pounds in 100 gallons.

† Mineral seal oil, 3/4 per cent.; figures in parentheses following oil refer to viscosity in seconds, SAYBOLT.

‡ A calcium caseinate spreader; 1/2 pound in 100 gallons.

exert a semisolvent action on the natural waxy coating, causing the lead particles to be more easily and firmly encompassed. After the disappearance of the spray oil, whether by volatilization or otherwise, the natural waxy constituents would then remain and form a more compact coating which would protect the arsenate particles against the solvent action of the cleaning fluid. Such action would be much more noticeable on varieties which are characterized by unusually high quantities of natural waxy coating, such as Arkansas Black and Esopus Spitzenburg.—K. S. MARKLEY AND CHARLES E. SANDO, *U. S. Department of Agriculture, Washington, D. C.*

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GAS INJURY TO PURE CULTURES OF *SPIRODELA*

An instance of severe injury to *Spirodela polyrhiza* (duckweed) growing in pure culture in a modified Knop's solution is here reported. This injury was caused by introducing small amounts of illuminating gas into the culture flask during transfer of the plants from one flask to another. The cultures were maintained in a sterile dilute Knop's solution, 250 cc. per 500-cc. flask, and were protected by a cotton plug over which was placed an inverted tall beaker (150 cc.). The latter was used to protect the stopper from dust, as it is advisable to have loose plugs for this type of work, on account of possible interference with gas exchange.

During the transfer of a single plant from one culture flask to another there was severe injury, not only to the transferred plant, but also to the culture from which the transfer had been made. The latter was sometimes lost entirely. The cotton was always flamed before removal, and again after it had been replaced. This was thought necessary because the cultures are often maintained for many weeks and are exposed to dust during that time. The interior of the beaker was flamed while held in an inverted position and was quickly placed over the cotton plug. The open neck of the flask was never flamed directly. Following this procedure the plants were almost always severely injured, as shown by abscission of the roots, separation of the plants into their component units, and death of nearly all the green portions except the region around the growing point at the node. After from one to two weeks the injured plants regenerated new leaves and roots, again producing normal colonies. Under these circumstances the intended experiments could not be made.

After some attempts to determine the cause of the injury, it was finally learned that unburned or partly burned gas was being introduced into the flask when the flamed inverted beaker was replaced over the neck of the flask. When flaming was omitted no injury whatever occurred, not even the loss of roots, which, in *Spirodela*, is a most sensitive indicator of injury.

Since flaming of the neck of sterile culture tubes and vessels is a procedure that has been much followed, although discouraged by many technicians, it is possible that occasional failure of growth in cultures is sometimes the result of this precautionary technique. Such injury would be more evident in those instances in which small numbers of individuals are transferred. It is possible that gas injury may have been encountered by others during the transfer of pure cultures of green plants or of bits of fungus mycelium.

Since *Spirodela* is sensitive to injury by fuel gas, an experiment was made to determine the relation of time of exposure to degree of injury. Pure cultures of vigorously growing plants were exposed to an atmosphere

consisting entirely of illuminating gas. This was a carburetted water gas with an illuminant content of 10.2 per cent. After exposure the gas was removed by a stream of air passed into the flask until no more odor was detected. Exposures were made for 5, 7, 8, and 10 minutes. Within less than an hour later severe injury could be detected in all cases. The leaves became a pale whitish green, indicating chlorophyll decomposition, the surface became slightly concave (in contrast with the normal slightly convex appearance), and numerous drops of liquid were exuded on the upper epidermis, most numerous about one-third the distance from the node to the tip. Later the roots separated from the plants. All plants after the 10-minute exposure were killed, and but few survived after a 5-minute exposure.

It is suggested that *Spirodela* may be used in a technique for detecting the presence of illuminating gas in soils, where high concentrations are often present as the result of leaks in gas mains. TRUE<sup>1</sup> has described a technique for this purpose, using the sweet pea. It is doubtful whether *Spirodela* is as sensitive to traces of illuminating gas or ethylene as those plants studied by CROCKER<sup>2</sup> and his coworkers.—ALBERT SAEGER, *Cornell University, Ithaca, New York*

#### OCCURRENCE OF DULCITOL IN *IRIDEAE LAMINARIOIDES* (RHODOPHYCEAE)

In studying the nature of the cell wall constituents of *Irideae laminarioides*, which grows abundantly on the rocks of the Pacific Coast, at Moss Beach, California, the investigation was primarily concerned with a polysaccharide, galactan, which consists chiefly of galactose units. The writer was also interested in determining whether or not the metabolism of this plant is based on a sugar such as glucose or sucrose, as in the higher plants, or on a carbohydrate other than these.

Material was collected at four different times of the year, on May 12, June 20, August 28, and October 27 of 1932. The plants were extracted with alcohol and analyzed for reducing and total sugars. All of the samples failed to give a reducing value with Fehling's solution either before or after hydrolysis. On concentrating the alcoholic extract, however, a thick syrup was obtained.

Since the presence of mannitol in brown algae was reported by KYLIN (5, 6) in 1913–1915, and more recently by HAAS and HILL (4), it seemed likely that this syrup might contain the alcohol. Its isolation was therefore

<sup>1</sup> TRUE, R. H. *Florists Exchange* 73: 13. 1930.

<sup>2</sup> CROCKER, WM. *Florists Exchange* 70: 15. 1929. Also in *Boyce Thompson Inst. Prof. Papers* 11: 81–85. 1929. And later publications in *Contrib. Boyce Thompson Inst.*

attempted. The plants were boiled for 15 minutes in 95 per cent. alcohol immediately after collection, then dried in a vacuum oven at 40° C. One hundred and fifty grams of the dry tissue were ground up and sieved. The ground material was then extracted in a large Soxhlet with 80 per cent. alcohol, and this extract was combined with the alcoholic portion in which the plants had been boiled immediately after collection. The combined solution was treated with lead acetate, the excess of lead removed with 2 per cent.  $\text{H}_2\text{SO}_4$ , and the lead sulphate filtered off. The filtered solution was then treated according to the method of HAAS and HILL (4) for preparation of mannitol, but no crystalline form of this alcohol could be obtained from the syrup. A portion of the syrup was placed in the vacuum oven and concentrated at 80° C. for 12 hours, the syrup then being weighed and its specific rotation observed. The substance showed no rotatory power; also, the addition of borax (1) to this solution did not increase its rotation. This fact furnished additional proof of the absence of mannitol.

Since the *Irideae laminarioides* contained a considerable amount of galactan, it was suspected that the sugar alcohol of galactan, dulcitol, might be present. The syrup was extracted again in a Soxhlet with absolute alcohol, the extract clarified with charcoal, concentrated to a syrup, and allowed to stand for about a week with occasional stirring. White crystals began to separate. After recrystallization of these crystals and examination, the following was observed: the specific rotation of this substance,  $[\alpha]_D = 0$ ; melting point 185°; oxidation with nitric acid yielded mucic acid. These facts constitute conclusive proof that this substance isolated from *Irideae laminarioides* was dulcitol.

HAAS and HILL (2, 3) have recently reported the occurrence of dulcitol and sorbitol in a red alga, *Brostrychia scorpoides*. On examination of eight other species of Rhodophyceae, however, they found no evidence of the presence of either dulcitol or sorbitol.

The isolation of dulcitol from *Irideae laminarioides*, therefore, confirms the occurrence of dulcitol in red algae found by HAAS and HILL.

The polysaccharide galactan isolated from *Irideae laminarioides* is precipitated out from 95 per cent. alcohol and is obtained in the form of threads. It absorbs many times its own weight of water and forms a colloidal solution. It gives no reducing value, but after hydrolysis with 2 per cent. sulphuric acid it strongly reduces Fehling's solution. Upon oxidation with nitric acid it yields mucic acid. This polysaccharide is being investigated at the present time.

It is conceivable that a possible equilibrium exists between the polysaccharide, galactan, and the sugar alcohol, dulcitol. This may be analogous in the carbohydrate metabolism of the plant to the equilibrium that exists

between starch and glucose in higher plants. This suggestion supports the idea that the metabolic process of some of the algae may depend upon carbohydrates other than glucose or sucrose. In the case of *Irideae laminarioides* this is probably dulcitol.—W. Z. HASSID, *University of California, Berkeley, Calif.*

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#### MOVEMENT OF ORGANIC MATERIALS IN PLANTS: A CORRECTION

In a recent note upon the mechanism of translocation,<sup>1</sup> the writers criticized the use made by CRAFTS<sup>2</sup> of the Poiseuille expression for uniform, non-turbulent, viscous flow in a capillary of known dimensions. To this criticism in all its general aspects we still adhere, especially in so far as it refers to the inapplicability of the formula in question to a "flow" which is clearly not uniform, and also to the comparison made between the whole phloem wall substance and the pores in the sieve plates as possible avenues for translocation. The basis of the latter criticism is that CRAFTS by treating the whole phloem wall substance as a single, circular capillary, derived pressures which can have no possible relation to the actual pressures involved in the production of a flow of the desired dimensions in the phloem wall. These are unjustifiably compared with pressures calculated to refer to flow through the pores in the sieve plates.

In the attempt to pursue CRAFTS' own method and insert a dimension (one half the mean wall thickness) which, on the assumption of flow

<sup>1</sup> STEWARD, F. C., and PRIESTLEY, J. H. Movement of organic materials in plants: A note on a recently suggested mechanism. Plant Physiol. **7**: 165–171. 1932.

<sup>2</sup> CRAFTS, A. S. Movement of organic materials in plants. Plant Physiol. **6**: 1–38. 1931.

through the whole wall, would lead to a more probable pressure an error has been made, an error which we have realized following correspondence with Dr. CRAFTS and which we now desire to correct. The treatment (p. 167) which actually led to a factor  $\frac{r}{(r_1)^4}$  by which CRAFTS' derived pressures should be multiplied ought to have led to the factor  $\frac{r}{(r_1)^2}$ . It will be clear from the context, however, and from the subsequent discussion (which points out that even thus modified the treatment cannot be accepted), that this error does not materially affect our general criticisms.—F. C. STEWARD and J. H. PRIESTLEY, *University of Leeds, England*.



## NOTES

**Annual Election.**—The results of the annual election of the American Society of Plant Physiologists have just been announced by the Secretary-Treasurer as follows: President for 1933-1934, DR. C. O. APPLEMAN, University of Maryland; Vice-President, DR. H. R. KRAYBILL, Purdue University; Secretary-Treasurer for 1933-1935, DR. A. E. MURNEEK, University of Missouri. The new officers inherit some difficult problems arising from the depression. In attempting to solve these problems, they will need and expect cooperation from the entire membership. The same optimism and courage that made possible the establishment of a good journal for publication of research will be able to conquer the present difficulties.

**Summer Meeting.**—The summer meeting of 1933 was held in Chicago on June 20-22, in connection with the programs of Sections G and O, A. A. A. S., the Botanical Society of America, the American Phytopathological Society, and the American Society of Agronomy. The special meeting for plant physiologists was a symposium on "Radiation and Plant Life" on June 22. Lively discussion of the papers featured the meeting, and enhanced the value of the conference.

**Boston Meeting.**—The tenth annual meeting of the Society will be held in Boston in December, 1933. The program will be arranged by a program committee, as usual. The chairman of this committee is Dr. B. E. GILBERT, Director of the Rhode Island Agricultural Experiment Station. Prompt response to requests of the committee will be greatly appreciated.

**Membership Committee.**—A large membership committee was appointed by President D. R. HOAGLAND, with Dr. LAURENZ GREENE, of Purdue University, as chairman. The October number of PLANT PHYSIOLOGY will be small, since it is necessary to utilize reserves in order to provide an October issue. Those who drop subscriptions during the depression are voting to discontinue the facilities for publication of research. At the same time, authors are more urgent about the appearance of their papers than in normal times. With more material on hand than can be used promptly, each member of the society should aid the membership committee to enlarge our funds for publication. Every new member helps just that much. Each fee pays for one page of printed matter. A few orders for back volumes would be valuable in providing larger funds for immediate use. It is not a question of whether one can afford to subscribe for journals at this time, but whether we can afford not to maintain the usual outlets for

publication of material. Research is without much value until it has been published and its results utilized by mankind.

**Purdue Section.**—The Purdue Section of the American Society of Plant Physiologists has just completed another successful year. There are 32 members, 11 of whom are members of the national organization. The average attendance has been about 25 at each biweekly meeting. Aside from the regular program, two dinner meetings were held. The first was a joint meeting with the Purdue Section of the American Chemical Society at which Dr. K. P. LINK, of the University of Wisconsin, gave an interesting discussion on the biochemistry of certain plant diseases. The speaker at the final meeting was Dr. I. L. BALDWIN, of the University of Wisconsin, who discussed the legume or root nodule bacteria.

The program for the regular meetings was as follows:

- November 7, Chemical and varietal studies of the consistency of canned pumpkin. D. M. DOTY.
- November 21, Separation and identification of viruses occurring in solanaceous plants. R. W. SAMSON.
- December 5, Virus diseases of various species of *Prunus*. J. A. MCCLINTOCK.
- January 16, Joint evening meeting with the Biological Society. Reports of the Atlantic City meeting of A. A. A. S.
- February 6, Some effects of light on the growth of asters. M. W. RICHMAN.
- March 6, Recent studies on cold tolerance of corn. J. R. HOLBERT, U. S. Department of Agriculture.
- March 20, Root development in hardy and non-hardy wheat varieties. W. W. WORZELLA.
- April 17, Effects of various salt solutions on respiration in seeds. W. W. JONES.

At the last regular meeting the following officers were elected for the year 1933-1934: Dr. G. N. HOFFER, Chairman; Mr. R. B. WITHROW, Secretary and Treasurer.—W. W. JONES, Secretary.

**International Critical Tables.**—An index has finally been provided for this important work. The price quoted to subscribers is \$3.00 for this volume. The McGraw-Hill Book Co. has rendered a great service to science in the publication of these tables, which are now rendered much more useful through careful indexing of the entire work in one volume.

**Handbook of Plant Analysis.**—The third volume of G. KLEIN's Handbuch der Pflanzenanalyse was published by the Vienna plant of Julius

Springer. It is an immense volume, 1613 pages, bound in two separate parts. The first half, 806 pages, contains methods of analysis for membrane substances, natural tannins, lichen acids, ethereal oils, caoutchouc and gutta-percha, and resins. The second half, 807 pages, is devoted to many types of glucosides (aliphatic and aromatics, flavones, anthocyanins, anthracenes, hydrocyanic, indoxyl, mustard oils, saponins, digitalis, and other obscure types), and pigments (carotinoids, chlorophyll, algal pigments, fungal and bacterial pigments, and other slightly studied pigments). An appendix treats lignin analysis. The index alone occupies 137 pages. It is not possible to describe the work in detail here. It is an extremely compendious handbook, and represents an enormous amount of labor on the part of the authors, editor and publishers. Orders may be sent to J. Springer, Schottengasse 4, Vienna I. The price of this volume in two parts is RM 162 unbound, RM 168 bound in cloth.

**Manual of Plant Biochemistry.**—Plant physiologists and biochemists will be interested to know that Dr. W. E. TOTTINGHAM is preparing a manual of plant biochemistry which will be published in a few months by the Burgess Publishing Co., 519-521 Second Ave. So., Minneapolis, Minnesota. Only a portion of the work has been examined, and no price can yet be quoted. Inquiries can be addressed to Mr. C. S. HUTCHINSON, of the Burgess Co.

**Microbiology of Forest Soils.**—The microflora and microfauna of forest soils are unique. A volume of D. FEHÉR, *Untersuchungen über die Mikrobiologie des Waldbodens*, has been published by J. Springer, Berlin. It contains eleven chapters, 272 pages. There is a table of contents, but no index. Methods of research are described, bacteria of forest soils, production of CO<sub>2</sub>, nitrogen cycle, seasonal changes in biological transformations, microbiological characteristics of sandy forest soils, fungi and algae in European forest soils, and protozoa of forest soils. An appendix deals with the special microbiological problems of alkali soils. The chapter on protozoa is by L. VARGA, and the alkali (Szik-Boden) soils are discussed by R. BOKOR. The work is a very valuable contribution, represents ten years of research in the Institute of which FEHÉR is the director, and brings much new information into brief compass. The price quoted is RM 24 for paper binding. Orders should be addressed to J. Springer, Linkstrasse 23-24, Berlin W9.

**Principles of Plant Physiology.**—A revised edition of this work by Dr. O. L. RABER has been published by Macmillan Co., at \$3.00 per copy. The general plan of the work remains unchanged. On the assumption that

we know more about aerobic respiration than anaerobic, the former is treated first, as a "known" from which to approach the "unknown." The facts of anaerobic respiration, however, are probably better substantiated than those of aerobic respiration, if we consider respiration as a chemical process. Students will find it a helpful introduction to plant physiology. Citations of recent literature have been liberally used. The book includes portraits of a number of living plant physiologists and plant chemists. Some errors that marred the first edition have been eliminated.

**Plant World in Florida.**—A very attractive book, the collection of the notes and writings of Dr. HENRY NEHLING, who died in 1929 after a long experience with Florida's flora, has been edited by ALFRED and ELIZABETH KAY. It carries a preface by DAVID FAIRCHILD, whose explorations were so delightfully presented to lovers of nature a couple years ago. Many groups of plants are described, always in alphabetic order within the group, so that it is easy to find the description and notes concerning any important member of the Florida vegetation. A fine portrait of Dr. NEHLING adorns the work as a frontispiece. The book was sponsored by the Garden Club of Palm Beach. The price is \$3.50 per copy, and orders for it can be sent to the publishers, Macmillan Co., New York.

**Plant Names.**—The Macmillan Co. has also published a little volume by Dr. LIBERTY HYDE BAILEY, on *How Plants Get Their Names*. Many botanists will be helped by this work, especially beginners who wish to understand the problems of nomenclature. It is written in very simple language, so that even a layman can grasp it. A long list of specific names with their pronunciations, and with their probable meanings in plain English, is a valuable feature of this handy little volume. It is quoted by the publishers at \$2.25 per copy.

# PLANT PHYSIOLOGY

OCTOBER, 1933

## CONCENTRATIONS OF INORGANIC IONS AS RELATED TO GROWTH OF EXCISED ROOT-TIPS OF WHEAT SEEDLINGS

PHILIP R. WHITE

(WITH SEVEN FIGURES)

### Introduction

Growth of excised wheat root-tips, as influenced by H-ion concentration of the medium, volume of the medium, temperature, illumination, and organic nutrition was considered in an earlier paper (10). The present contribution deals with additional preliminary experiments on growth of the same kind of root-tips as related to concentrations and proportions of inorganic ions (other than  $H^+$  and  $OH^-$ ) in the medium.

Except as otherwise stated, experimental procedures were like those described in the paper just referred to: the culture flasks (each with a single root-tip) stood in a well ventilated basement room, with temperature maintained at about 25° C. and with continuous artificial illumination from nitrogen-filled Mazda "daylight" lamps; the standard nutrient medium used was the USPENSKI solution (9), to each liter of which 20 gm. of

TABLE I  
CONCENTRATION OF SALTS USED

SALT	CONCENTRATION		
	MOLAR	NORMAL	MG. PER LITER
$Ca(NO_3)_2$ .. . . .	0.0006	0.0003	100
$MgSO_4$ .. . . .	0.0002	0.0001	25
$K_2CO_3$ .. . . .	0.00025	0.000125	35
$KNO_3$ ....	0.00025	0.00025	25
$KH_2PO_4$ .. . . .	0.000185	0.000061	25
$Fe_2(SO_4)_3$ .. . . .	0.000003	0.000001	1.25

dextrose was added, with the extract of 0.1 gm. of dry brewers' yeast. The salts of the USPENSKI solution, together with their molar and normal concentrations and their concentrations in milligrams per liter, are shown in table I.

The total salt concentration was consequently about 0.0015 M., or 211.25 mg. per liter. Ionization is supposed to have been practically complete. Neglecting such small amounts of incompletely ionized salts as may have been present, as well as traces of unknown impurities in the chemicals used and unknown constituents of the yeast extract added, the standard medium may be taken to have contained, besides  $H^+$  and  $OH^-$ , the following potential ions in approximately the partial concentrations shown.

TABLE II\*  
CONCENTRATION OF IONS IN SOLUTION

		CONCENTRATION PER LITER (MILLIGRAMS)		
		Mg.-ION	Mg. EQUIVALENT	Mg.
Cations	K . . . . .	0.940 ( $U_K$ )	0.940	36.70
	Ca . . . . .	0.600 ( $U_{Ca}$ )	0.300	24.00
	Mg . . . . .	0.200 ( $U_{Mg}$ )	0.100	5.00
	Fe . . . . .	0.006 ( $U_{Fe}$ )	0.002	0.35
Anions	$NO_3$ . . . . .	1.45 ( $U_{NO_3}$ )	1.450	91.0
	$SO_4$ . . . . .	0.21 ( $U_{SO_4}$ )	0.100	20.0
	$CO_3$ . . . . .	0.25 ( $U_{CO_3}$ )	0.125	15.0
	$PO_4$ . . . . .	0.18 ( $U_{PO_4}$ )	0.090	17.5

\* In table II and throughout this paper, the symbols for the several kinds of ions appear without their plus or minus signs; thus K is used instead of  $K^+$ , Fe is used instead of  $Fe^{+++}$ ,  $SO_4$  instead of  $SO_4^-$ , etc. Such usage avoids considerable expense in printing and cannot lead to ambiguity in such discussions as this.

In table II, each partial concentration is shown by three different values, each of which might be computed from either of the others: (1) "Milligram-ion per liter" is the quotient obtained by dividing the number of milligrams per liter, of the ion in question, by the ionic weight of the latter; it corresponds to the familiar "gram-molecules per liter." (2) "Milligram-equivalent per liter" is the quotient obtained by dividing "milligram-ion per liter" by the valence of the ion in question; it corresponds to the familiar "normal concentration." (3) "Milligrams per liter" is simply the weight, in milligrams, of the ion in question contained in a liter of medium; its value is identical with that of the corresponding concentration expressed as parts per million, being the corresponding percentage concentration multiplied by 10,000. Relative ionic concentrations rather than

relative molar concentrations or relative weights of elements, etc., per liter, are of primary interest in comparing nutrient media with respect to their physiological influence, as was emphasized in SHIVE's discussion of ionic ratios (7); therefore the concentration values considered in this paper are always ionic. They are generally referred to the mg.-ion values just given, which serve as concentration units. Convenient designations for these units are shown in parentheses in table II; for example, 0.94 mg.-ion of K per liter (the standard partial concentration of K) is called  $U_K$ , 1.45 mg.-ion of  $NO_3$  per liter is called  $U_{NO_3}$ , etc. Consequently  $0.5 U_{NO_3}$  represents 0.725 mg.-ion of  $NO_3$  per liter, etc.

With the standard medium as a basis, various modifications were tested, these being made up with different concentrations of the ions considered. There were 127 of these modified media, giving a great number of differences in ion proportions. The influences exerted on growth by some of the main ions of the standard solution were thus studied separately or in pairs, as will be seen from the account of results. Each ion studied was employed at higher and lower concentrations than were specified for the standard medium, which was always used as control.

The complete omission of an ion in the preparation of a medium did not, of course, imply that the ion in question was entirely absent, for very small traces of all the main ions may be supposed to have been derived from salt impurities or from the yeast extract used. Media designated as "without K," for instance, probably had unknown but very small concentrations of K.

To study the ion relations of an organism it would naturally be desirable, among other things, to alter the concentration of each ion separately, without at the same time introducing any other alteration in the constitution of the nutrient medium; but this is obviously impossible, since ions cannot be handled separately, as is possible with molecules. In order to alter the concentration of any ion it is necessary to alter the concentration of one or more of the other ions also,—an obvious consideration which complicates matters exceedingly, although it has seldom been taken seriously into account in solution-culture experimentation (see MORITA and LIVINGSTON's (5) plan for studying solution cultures of wheat plants in solutions from which potassium was omitted). In the present experiments on excised wheat root-tips, the concentration of any ion was decreased by suitably decreasing the concentration of its salt or salts and by adding a suitable amount, either of a sodium salt with the corresponding anion or of a chloride with the corresponding cation; and the concentration of any ion was increased simply by adding to the solution the requisite amount either of the corresponding sodium salt or of the corresponding chloride. By this procedure each modified medium was approximately like the standard medium with respect to the concentrations of all the ions of the latter, excepting the ion or ions

whose partial concentrations were purposely altered; but some of the modified solutions differed from the standard solution also with respect to their concentration of Na or of Cl, for these extra ions are not included in the USPENSKI solution. For the purposes of this preliminary study, it may be tentatively supposed, without prejudice, that these small additions of Na or of Cl were not significantly influential upon the growth of the root-tips.

Each culture flask, with 50 ml. of medium, had a single root-tip. The root-tips were measured after a period of 14 days, the length of the main root and the total length of all branches being recorded for each culture.

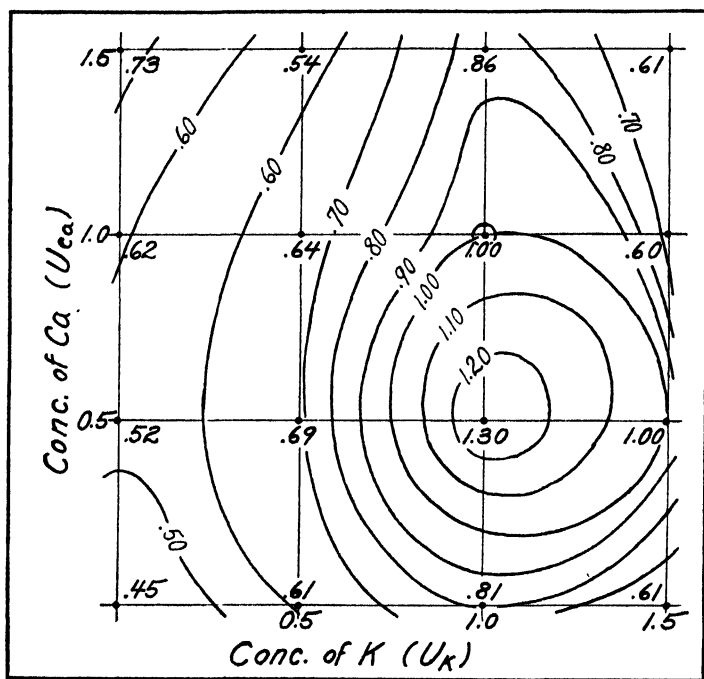


FIG. 1. Three-dimensional diagram showing by means of isopleths growth responses as related to different concentrations of K and Ca. Relative growth indices are inscribed at the points representing the several combinations of these ions.

The sum of these measurements gave the total increment of elongation for the culture in question. (Because the original excised tips, with which all cultures were started, were uniformly only about 3 mm. long, this original length may be considered as insignificant.) The growth increments for all like cultures in each series were averaged, to give a single numerical growth index for the particular solution employed. The relative physiological suitability of any medium tested may be judged by comparing its growth index with the corresponding growth index for the standard medium. Photo-

graphs of representative root-tips were made in many instances and records were kept as to general appearance and amount of branching.

## Experimental results

### CATIONS

*The influence of K and Ca upon growth* was studied in two similar series of cultures, carried out at about 26° C. Besides the standard medium there were 15 modifications, and each series comprised 10 cultures of each medium. The modified media were like the standard medium in ionic concentrations, excepting with respect to the concentrations of K and Ca (and, in

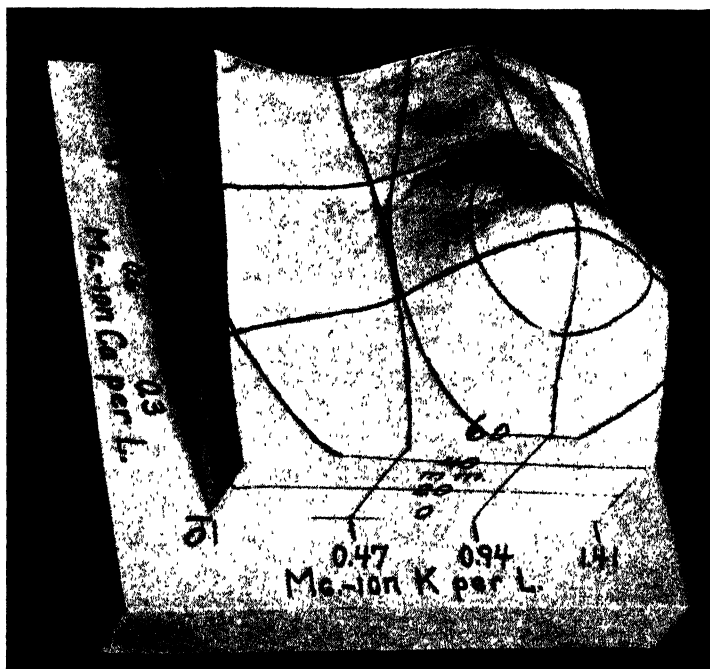


FIG. 2. Model showing the relations in figure 1, but concentrations are shown here as mg.-ions per liter and altitudes are actual growth indices, in mm.

some instances, with respect to added Na or Cl). The replacements were:  $K_2CO_3$  by  $Na_2CO_3$ ,  $KNO_3$  by  $NaNO_3$ ,  $KH_2PO_4$  by  $NaH_2PO_4$ , and  $Ca(NO_3)_2$  by  $NaNO_3$ .  $KNO_3$  was supplemented by KCl, and  $Ca(NO_3)_2$  was supplemented by  $CaCl_2$ . The concentrations of K and Ca in these series are shown on the diagram of figure 1. One medium received neither K nor Ca, three received no K, and three received no Ca. Those receiving K had K-ion concentration of 0.5  $U_K$ , 1.0  $U_K$ , or 1.5  $U_K$ , while those receiving Ca had Ca-ion concentration of 0.5  $U_{Ca}$ , 1.0  $U_{Ca}$ , or 1.5  $U_{Ca}$ .

The first of these two series was carried out in continuous darkness. The pH values of the media at the end of the culture period were all between 5.4 and 5.7, a range previously shown (10) to give excellent growth of these root-tips. The growth indices obtained, expressed in terms of the index for the standard medium (76.0 mm. = 1.00), are inscribed at the points on the diagram just mentioned. Each isopleth thus represents a series of K-Ca ratios that were about equally effective for growth of these root-tips. The solid diagram of figure 2 represents these results in another way.

Best growth occurred in the medium having a K concentration of  $U_K$  (i.e., 0.94 mg-ion per liter) and a Ca concentration of  $0.5 U_{Ca}$  (i.e., 0.30 mg-ion per liter). It thus appears that the standard medium might have given greater growth had its concentration of Ca been somewhat lower, but that its concentration of K was about optimal for the range of salt proportions represented by this experiment.

It is obvious that if a set of vertical or horizontal profiles were erected upon the diagram of figure 1, the growth relations shown by any profile might be very different from those shown by another profile of the same set. Any such profile is, of course, the concentration-growth curve as usually constructed when an ion is treated as an independent variable, and the failure of these curves to coincide under experimental complexes that differed only slightly and with respect to only one other variable emphasizes the necessity of considering all the effective variables in such studies as this.

In the second of these two series with K and Ca, carried out in diffuse natural daylight, the eight media represented by the points on the lower half of figure 1 gave results that agreed essentially with those obtained from the first series. The best growth, for the same medium as before, showed an absolute index value about 15 per cent. lower than in the first series. The final pH values of these media were between 5.2 and 5.6, about as in the first series. On the other hand, the eight media represented by the points on the upper half of the figure were found (for some unknown reason, perhaps connected with autoclaving) to have final pH values as high as 7.2, being thus much more alkaline than the corresponding media of the first series. The growth indices for these more alkaline media were much lower than those for media similar in constitution but with more acid reaction, as shown on the figure,—an observation which is in accord with an earlier conclusion (10) that these root-tips gave only relatively low growth indices when the pH value of the medium was higher than about 6.0. This unexplained difference in final pH values between two sets of media intended to be alike seems to emphasize the need for extreme care in the preparation and autoclaving of culture media for studies of this kind. These media were apparently very labile under some of the conditions to which they were subjected.

*The partial concentration of Mg was varied* in six series of cultures. Two of these were carried out in darkness, one in continuous weak artificial light, and three in more intense continuous artificial light such as was employed in earlier experiments on light influence (10). The first three had Mg concentrations of 0.0, 0.5, 1.0, and 1.5  $U_{Mg}$ , and the last had concentrations of 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5  $U_{Mg}$ . The partial concentrations of the other ions were always the same as those specified for the standard medium (excepting Na and Cl in some cases). The medium without Mg contained  $Na_2SO_4$  instead of  $MgSO_4$ ; the media with Mg concentrations lower than 1  $U_{Mg}$  contained both  $MgSO_4$  and  $Na_2SO_4$ ; and those with Mg concentrations higher than 1  $U_{Mg}$  received additional Mg as  $MgCl_2$ .

The average growth index for all tests in which Mg was omitted was 45 per cent. greater than the corresponding average for the standard medium. When Mg was present at a concentration of 0.5  $U_{Mg}$ , the average index was somewhat (8 per cent.) smaller than the corresponding control average, but this may not be a significant difference. With Mg concentration of 1.5 or 2.0  $U_{Mg}$ , the respective averages for all series were 12 or 13 per cent. greater than the control average. With Mg concentration of 2.5  $U_{Mg}$ , the average for all series was sensibly like the control average. Of course there was some deviation among the several series, but the detailed data generally indicate more growth in the medium without Mg than in any of the others. The relative growth indices for the medium without Mg in these experiments are as follows, the corresponding control index being taken as 1.00: For two series in continuous darkness, 0.89 and 1.18; for one series in weak continuous artificial light, 1.08; for three series in more intense continuous artificial light, 2.45, 1.48, and 1.63. A representative of the root-tips grown without Mg is shown at the left in figure 3; these were straight and of unusually great length, but generally only sparsely branched. A comparable representative root-tip grown in standard medium with Mg concentration of 1  $U_{Mg}$  is shown at the center of the figure; these control root-tips were much shorter and more crooked but had more branches than those grown without Mg. The right-hand root-tip in the figure represents those grown with an Mg concentration of 2  $U_{Mg}$ ; their growth habit was much like that of those grown without Mg, but their growth index is 49 per cent. less. All three of the root-tips were grown with temperature about  $26^\circ$  and with relatively intense continuous artificial illumination, the only difference in treatment being with respect to Mg concentration.

The medium without Mg was somewhat opalescent when taken from the autoclave, but precipitation soon ensued, leaving the liquid clear and colorless. On the other hand, all solutions with Mg were clear when taken from the autoclave, and they showed no subsequent precipitation but were somewhat yellowish in color, suggesting that some caramelization of dextrose may

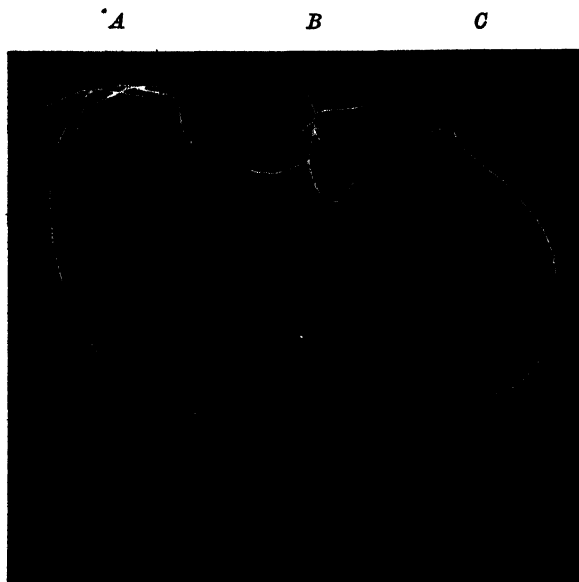


FIG. 3. Representative root-tips grown 2 weeks in media differing only with respect to the concentration of Mg: *A*, without Mg; *B* and *C*, with 0.2 mg.-ion of Mg per liter (standard) and 0.4 mg.-ion of Mg per liter respectively. About natural size.

have occurred in the process of autoclaving. This difference between the medium without Mg and those with Mg was not paralleled by any significant difference in pH value. The yellow tint of the media with Mg was found to be progressively more pronounced as the concentration of  $K_2CO_3$  was higher. These observations led to the suggestion that a medium with a concentration of  $CO_3$  less than  $U_{CO_3}$  might perhaps give more vigorous growth than was given by the standard medium.

*The concentrations of both Mg and  $CO_3$  were therefore varied*, in a single experiment which was carried out at about  $24^\circ$  and with continuous intense artificial illumination. Besides the standard medium there were in this series 17 modified media, and each of the 18 was represented by 10 cultures. The concentrations of all component ions excepting Mg and  $CO_3$  (and in some cases Na and Cl) were the same for all these media. The 18 different combinations of Mg concentrations and  $CO_3$  concentrations tested are shown in figure 4, where it is seen that Mg had concentrations of 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5  $U_{Mg}$ , and that  $CO_3$  had concentrations of 0.0, 1.0, and 2.0  $U_{CO_3}$ . The media from which  $CO_3$  was omitted received KCl instead of the carbonate; those with  $CO_3$  at concentrations lower than 1  $U_{CO_3}$  received both  $K_2CO_3$  and KCl; and those with  $CO_3$  concentrations higher than 1  $U_{CO_3}$  received both  $K_2CO_3$  and  $Na_2CO_3$ .

The relative values of the growth indices obtained from this series are inscribed on the 3-dimensional diagram of figure 4, in which each contour line or isopleth indicates concentration combinations (Mg-CO<sub>2</sub> ratios) that gave like growth values. The hachured area represents media closely approaching the standard medium in effectiveness. The actual average index for the standard medium was 72.0 mm., which is taken as unity.

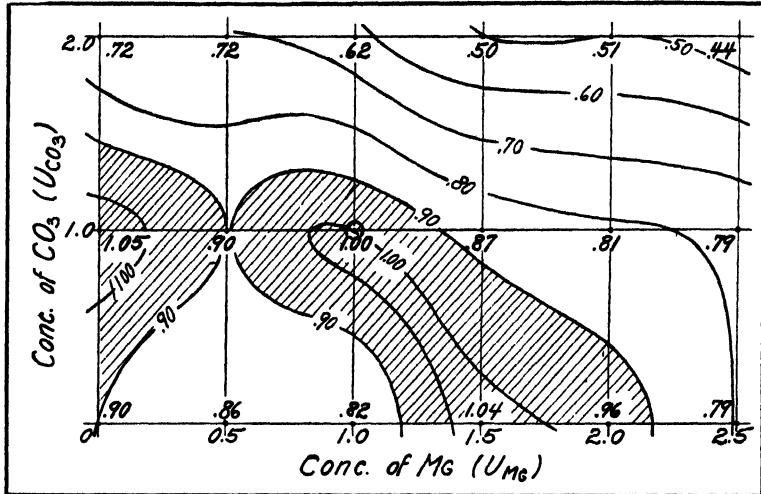


FIG. 4. Three-dimensional diagram showing by means of isopleths growth responses as related to different concentrations of Mg and CO<sub>2</sub>; relative growth indices are inscribed at the points representing the several combinations of these ions. Hachured area represents media closely approaching the standard medium (1.00) in effectiveness.

With the exception of the single point whose coordinates are  $0.5 U_{Mg}$ ,  $1 U_{CO_3}$ , the relations shown seem to be satisfactorily consistent; perhaps the growth index for the medium represented by that point may be erratically low. At any rate it appears that the standard medium was slightly surpassed, for growth in these 2-week tests, by two modified media, one without Mg (and with  $CO_3$  concentration of  $1 U_{CO_3}$ ) and the other without  $CO_3$  (and with Mg concentration of  $1.5 U_{Mg}$ ). The standard medium might apparently be simplified and somewhat improved by omitting Mg or by omitting  $CO_3$  and at the same time increasing the Mg concentration by 50 per cent.

These suggestions may be added to the suggestion that the standard medium might be improved by decreasing its Ca concentration by 50 per cent. But it is to be remembered that the physiological influences due to the several ions are not to be regarded as independent variables; consequently it need not be supposed that the standard medium would be improved by simultaneously decreasing its Ca concentration and omitting Mg, or by

simultaneously decreasing its Ca concentration, increasing its Mg concentration, and omitting  $\text{CO}_3$ . A more elaborate series of tests than has thus far been attempted would be required to throw light on the question thus raised. In this connection it is to be borne in mind that such modifications of the standard medium would each necessarily involve still other resultant modifications with respect to the concentrations of ions other than those primarily considered.

*Three different sources of iron* were compared in five series of cultures with temperature of  $22^\circ$ – $27^\circ$ . The substances used were  $\text{Fe}_2(\text{SO}_4)_3$  (the iron salt specified in the USPENSKI formula),  $\text{Fe}_2\text{Cl}_6$ , and a commercial preparation of uncertain composition called "iron citrate."<sup>1</sup> Ferric sulphate is not deliquescent, and specified concentrations of it may be prepared by simple weighing without special precautions; but it tends to form ferric hydroxide in solution, which is only slightly soluble. Ferric chloride, which has been used as iron source by many students of plant nutrition, is markedly deliquescent and it is usually undesirable to depend on weighed quantities of it for specified concentrations. Of course, measured quantities of a solution whose Fe content has been ascertained by analysis may be used in preparing nutrient media. In complex nutrient solutions, such as those of this study, each of these sources of iron may give concentrations of Fe that are markedly different from values computed on the basis of weighing and the formula, especially when the solutions are not decidedly acid. Despite these and similar troublesome considerations, the solutions used in these experiments were prepared in the ordinary way, by weighing, on the supposition that the three formulas were  $\text{Fe}_2(\text{SO}_4)_3$ ,  $\text{Fe}_2\text{Cl}_6 \cdot 12\text{H}_2\text{O}$ , and  $\text{FeC}_6\text{H}_5\text{O}_7 \cdot 3\text{H}_2\text{O}$ . Influences on growth that might possibly be exerted by the very low concentrations of the anions of these salts were left out of account. All the constituents of these solutions, except the iron source, were at the respective concentrations specified for the standard medium. The Fe concentrations employed were 0.0, 0.5, 1.0, and 1.5  $\text{U}_{\text{Fe}}$  ( $\text{U}_{\text{Fe}}$  being 0.006 mg.-ion per liter).

<sup>1</sup> The U. S. Pharmacopoeia, 7th ed. (6), gives "iron citrate" as  $\text{FeC}_6\text{H}_5\text{O}_7 \cdot 3\text{H}_2\text{O}$  [?], after the formula of SCHIFF, while BELLSTEIN (1) gives forms with 1,  $1\frac{1}{2}$ , and 3 molecules of water of crystallization as well as more complex formulas. BELLONI (2), who gave special attention to this material, stated that it is probably a dicitric-triferric citrate of the base  $[\text{Fe}_2(\text{C}_6\text{H}_5\text{O}_7)_2(\text{OH})_2]\text{OH}$ , with the formula  $[\text{Fe}_2(\text{C}_6\text{H}_5\text{O}_7)_2(\text{OH})_2(\text{OH}_2)_2]\frac{1}{2}\text{C}_6\text{H}_5\text{O}_7 \cdot 6\text{H}_2\text{O}$ . Among physiologists who have employed iron in citrate form, HOPKINS (3) used  $\text{KC}_6\text{H}_5\text{O}_7$  and  $\text{FeCl}_3$  together in his nutrient solution. He gives the formula of the resulting potassium-iron compound as  $\text{KH}_2[(\text{C}_6\text{H}_5\text{O}_7)(\text{CO}_2)_2\text{Fe}_2]$ , and states that he is "of the opinion that any preparation of ferric citrate purchased on the market would be of rather uncertain composition" (3a). A letter from the Chemical Division of the Eastman Kodak Co. refers to ferric ammonium citrate (which is used in photography) as  $4\text{FeC}_6\text{H}_5\text{O}_7 \cdot 3(\text{NH}_4)_2\text{C}_6\text{H}_5\text{O}_7 \cdot 3\text{Fe}(\text{OH})_3$ .

Three series, one for each iron source, were carried out simultaneously, each series having 10 cultures with each Fe concentration. The cultures received only diffuse natural daylight. Relative values of the resulting growth indices are shown below. The actual average index for the standard medium (marked with an asterisk) was 64.4 mm., which is taken as unity. The medium without Fe gave an actual average index of 48.8 mm., or a relative index of 0.74, which is lower than any of the nine index values for solutions containing Fe.

FE SUPPLIED AS	FE CONCENTRATION (MG.-ION PER LITER)		
	0.003	0.006 ( $U_{Fe}$ )	0.009
$Fe_2(SO_4)_3$ . . .	0.80	1.00*	1.48
$Fe_2Cl_6$ . . . . .	0.92	0.91	0.59
"Ferric citrate" . . . . .	0.89	1.11	0.94

Only with the sulphate were the growth values regularly larger as the Fe concentration was higher. With Fe concentration of 0.003 mg.-ion per liter the chloride and the "citrate" were apparently both slightly superior to the sulphate. With Fe concentration of 0.006 mg.-ion per liter ( $U_{Fe}$ ), the citrate was apparently slightly, although probably not significantly, superior to the sulphate and the chloride was apparently somewhat inferior to the sulphate. With Fe concentration of 0.009 mg.-ion per liter, both chloride and citrate were very much inferior to the sulphate. The indications are that these three sources of iron were about equally efficient when the Fe concentration was 0.5  $U_{Fe}$  or 1  $U_{Fe}$ , but that the citrate was unsatisfactory and the chloride was markedly toxic when the Fe concentration was 1.5  $U_{Fe}$ .

The series with  $Fe_2(SO_4)_3$  was repeated, with results almost exactly like those just stated for cultures in media containing iron in this form. Another like series with  $Fe_2(SO_4)_3$ , carried out in continuous darkness, gave an average growth index of 87.8 mm. for the standard medium. The relative growth indices were as follows:

WITHOUT FE	WITH FE (MG.-ION PER LITER)		
	0.003	1.006 (1 $U_{Fe}$ )	0.009
0.65 . . . . .	0.84	1.00	1.14

Neither the chloride nor the citrate appears to have given any considerable promise as a source of iron in cultures of this sort. It is equally clear that  $Fe_2(SO_4)_3$  was an excellent source of iron, giving progressively greater growth as the Fe concentration was higher; also that the standard medium

(with 0.006 mg.-ion of Fe per liter) was markedly deficient in Fe for these root-tips. Representative samples of root-tips grown without Fe and with these three different concentrations of  $\text{Fe}_2(\text{SO}_4)_3$  are shown by the photographs of figure 5. It is obvious that the highest Fe concentration tested

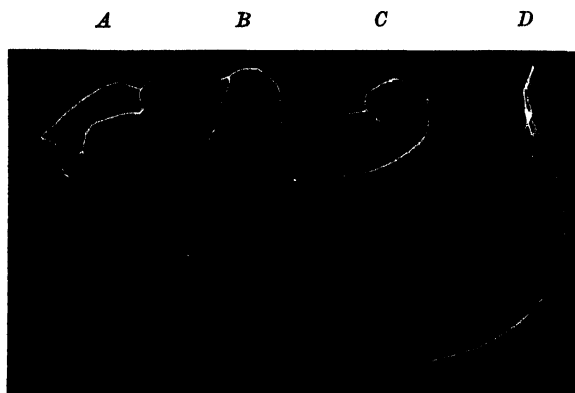


FIG. 5. Representative root-tips grown 2 weeks in media that differed only with respect to the concentration of Fe as  $\text{Fe}_2(\text{SO}_4)_3$ : A, without Fe; B, C, and D, with 0.003, 0.006 (standard), and 0.009 mg.-ion of Fe per liter respectively. About 0.6 natural size.

(0.009 mg.-ion per liter) gave by far the best growth, while the media with Fe concentration of 0.003 or 0.006 mg.-ion per liter gave relatively little growth, being only slightly superior to the medium without iron.

#### ANIONS

*The effects of omitting any three of the four main anions* of the standard medium were tested in a series comprising four modified media besides the standard one. These experiments were carried out at about 25° C., in continuous weak artificial light, and there were 20 cultures for each medium. Each of the four modified media was like the standard one with respect to dextrose, yeast extract, all the cations of the USPENSKI formula,  $\text{PO}_4$ , and  $\text{Fe}_2(\text{SO}_4)_3$ ; and each contained, in addition, either  $\text{NO}_3$ , Cl,  $\text{SO}_4$ , or  $\text{CO}_3$ , making in each case the total anion concentration like that of the standard medium. According to the anion that predominated in each, these four modified media may be named the nitrate medium, the chloride medium, the sulphate medium, and the carbonate medium. It will be observed that, aside from possible impurities in the salts used, and aside from the yeast extract used, the nitrate medium contained no Cl or  $\text{CO}_3$ , and only the very small amount of  $\text{SO}_4$  corresponding to 0.006 mg.-ion per liter as  $\text{Fe}_2(\text{SO}_4)_3$ ; and that neither of the three remaining modified media contained any nitrate, etc. These modified media were prepared according to the specifications for the standard medium but with the following substitutions: For the nitrate

medium  $\text{MgNO}_3 \cdot 6\text{H}_2\text{O}$  was used instead of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  and  $\text{KNO}_3$  was used in place of  $\text{K}_2\text{CO}_3$ . For the chloride medium  $\text{CaCl}_2$  was used instead of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  was used instead of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and  $\text{KCl}$  was used in place of both  $\text{KNO}_3$  and  $\text{K}_2\text{CO}_3$ . For the sulphate medium  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$  was used instead of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$  and  $\text{K}_2\text{SO}_4$  was used in place of both  $\text{KNO}_3$  and  $\text{K}_2\text{CO}_3$ . For the carbonate medium  $\text{CaCO}_3$  was used instead of  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ,  $3\text{MgCO}_3 \cdot \text{Mg}(\text{OH}) \cdot 3\text{H}_2\text{O}$  was used instead of  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and  $\text{K}_2\text{CO}_3$  was used in place of  $\text{KNO}_3$ . Both after autoclaving and at the end of the experiment, all but the carbonate medium showed pH values between 5.4 and 5.7 and no precipitation was observed. The carbonate medium was more alkaline, with a pH value of 5.95, and it showed some precipitation.

The relative values of the growth indices derived from this series are as follows, the actual value for the standard medium being 103 mm.:

Standard medium	1.00
Nitrate medium (with $\text{NO}_3$ and $\text{PO}_4$ but practically without either $\text{Cl}$ , $\text{SO}_4$ , or $\text{CO}_3$ )	0.87
Chloride medium (with $\text{Cl}$ and $\text{PO}_4$ but practically without either $\text{NO}_3$ , $\text{SO}_4$ , or $\text{CO}_3$ )	0.73
Sulphate medium (with $\text{SO}_4$ and $\text{PO}_4$ but practically without either $\text{NO}_3$ , $\text{Cl}$ , or $\text{CO}_3$ )	0.55
Carbonate medium (with $\text{CO}_3$ and $\text{PO}_4$ but practically without either $\text{NO}_3$ , $\text{Cl}$ , or $\text{SO}_4$ )	0.32

Root-tips grown in the standard medium were apparently in excellent condition, with an average of 7 branches each. Those grown in the nitrate medium appeared to be in very good condition but they were more slender than those grown in the standard medium; they had, on the average, 6 branches each. Root-tips grown in the chloride medium were generally shorter than those grown in the standard medium or in the nitrate medium, although they had, on the average, 8 branches each; both main root and branches were remarkably thick and stubby, with somewhat swollen tips. Root-tips grown in the sulphate medium were small but their average number of branches was eight and they had much the same appearance as those grown in the standard medium, excepting with respect to size. Those grown in the carbonate medium were clearly in very poor condition, crooked and swollen, and with an average of only 3 branches each, the branches being very short and stubby.

Representatives from these five media are shown in the photograph of figure 6. The fact that every one of the four modified media gave considerable growth indicates that vitality was maintained for the 2-week period in spite of these great chemical modifications of the medium. That the three

modified media without  $\text{CO}_3$  all have good, or at least fair, growth, while the carbonate medium gave very poor growth, appears to support the suggestion, already made, that  $\text{CO}_3$  might well be omitted from media of this general type for the plant material and period length used in this study. The omission of  $\text{Cl}$ ,  $\text{SO}_4$ , and  $\text{CO}_3$  together (nitrate medium) resulted in a growth value 87 per cent. as large as was obtained with the complete standard medium, from which it appears that  $\text{Cl}$  and  $\text{SO}_4$ , as well as  $\text{CO}_3$ , were unessential to good growth.

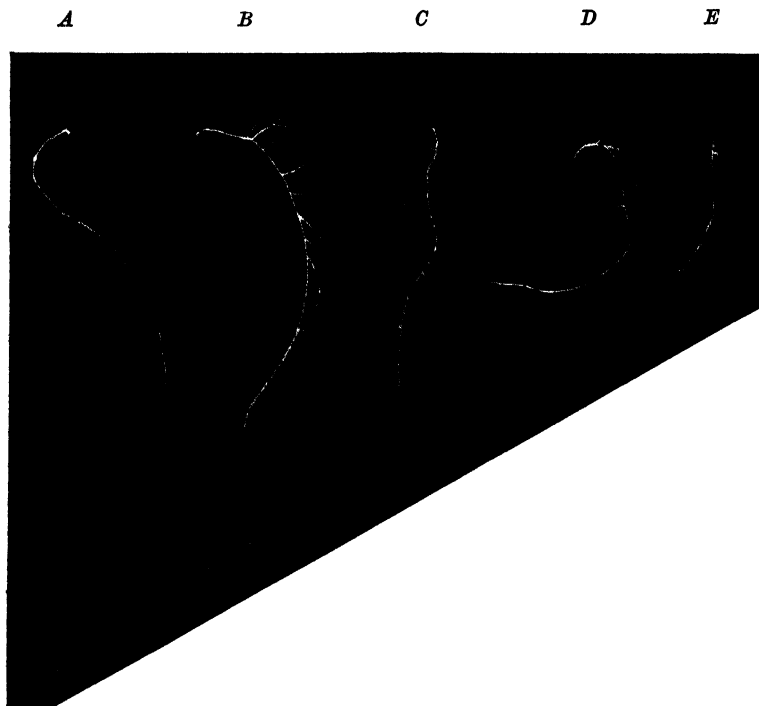


FIG. 6. Representative root-tips grown 2 weeks in media: *A*, standard in all respects; *B*, like *A* but with  $\text{SO}_4$  and  $\text{CO}_3$  replaced by  $\text{NO}_3$ ; *C*, like *A* but with  $\text{NO}_3$ ,  $\text{SO}_4$ , and  $\text{CO}_3$  replaced by  $\text{Cl}$ ; *D*, like *A* but with  $\text{NO}_3$  and  $\text{CO}_3$  replaced by  $\text{SO}_4$ ; *E*, like *A* but with  $\text{NO}_3$  and  $\text{SO}_4$  replaced by  $\text{CO}_3$ . About natural size.

Of the four anions here considered,  $\text{NO}_3$  was obviously the most important for extensive growth, but the good or medium growth obtained with the chloride medium and with the sulphate medium indicates that even  $\text{NO}_3$  was not essential for growth in these 2-week tests. Without  $\text{NO}_3$  a relative growth index as great as 0.73 was obtained, which is surprising in view of the great importance generally assigned to nitrates in the nutrition of higher green plants, and in view of the generally accepted statement that such organisms are unable to utilize molecular nitrogen (see LIPMAN and TAYLOR

(4), however). It is of course logically possible that nitrification occurred in the sulphate medium and in the chloride medium, through the action of nitrifying bacteria, but there is no direct evidence favoring such a supposition. A considerable but surely very small amount of  $\text{NO}_3$ , may have been introduced into the media planned to be without  $\text{NO}_3$ , in the yeast extract, and as possible impurities in the dextrose used or in the salts employed. It is also logically if only remotely possible that the original 3-mm. root-tips may have carried a sufficient amount of nitrogenous compounds to maintain good growth for two weeks, but cultures in the chloride medium gave final total lengths about 25 times as great as the original length (3 mm.), and those in the sulphate medium gave final total lengths about 19 times as great as the original length. Root-tips grown in the chloride medium or in the sulphate medium formed, on the average, 8 new growing points (branch roots). If one of these tips 3 mm. long contained sufficient assimilated or assimilable nitrogen to support such pronounced enlargement, and such multiplication of meristematic tissue (without any apparent degeneration of the older and basal tissues), that fact should be noteworthy.

*The nitrate ion and the ammonium ion were compared*, as to their influence on the growth of these root-tips, by means of a number of experiments with media that were like the standard one in regard to all features excepting the nitrogen source but different from the standard medium and among themselves in that respect. Several concentrations of  $\text{NO}_3$  and its omission, and the substitution of  $\text{NH}_4\text{Cl}$  for  $\text{Ca}(\text{NO}_3)_2$  and  $\text{KNO}_3$  were thus tested. There were 10 cultures for each  $\text{N}_2$  concentration, and cultures were carried out both in continuous darkness and in continuous weak artificial light, the temperature being about  $26^\circ$ . The presence of both  $\text{NO}_3$  and  $\text{NH}_4$  together

TABLE III

$\text{NO}_3$	$\text{NH}_4$	TOTAL $\text{N}_2$	RELATIVE GROWTH INDEX
<i>mg.-ion per l.</i>	<i>mg.-ion per l.</i>	<i>mg. per l.</i>	
0.0	0.0	0.0	1.04
0.0	0.32	4.5	0.93
0.0	0.64	9.0	0.98
0.45	0.0	9.8	1.09
0.56*	0.0*	12.0*	1.00
0.0	0.96	13.5	0.95
0.0	1.28	18.0	1.00
0.90	0.0	19.5	1.00
0.0	1.60	22.5	1.01
1.35	0.0	29.3	1.00

\* Standard medium.

in the same medium was not tested. All of these tests gave results sensibly like those obtained with standard medium. Table III shows the nature and concentration of the nitrogen source used in ten of these modified media, together with the relative growth index obtained with each modification. With the standard medium (marked by asterisks), excellent growth was obtained and the actual average index was 66.4 mm., which is taken as unity.

In these tests it apparently made no significant difference whether  $\text{NO}_3$  or  $\text{NH}_4$  was purposely present or absent, or whether either of these nitrogen sources was used at low or at high concentration. This strengthens and extends the conclusions reached from the experimental series previously described.

*Effects of modification of the  $\text{PO}_4$  concentration of the medium* were tested in three series. The temperature varied between  $22^\circ$  and  $26^\circ$ . In two series constituents other than  $\text{KH}_2\text{PO}_4$  were maintained at the concentrations specified for the standard medium and the  $\text{PO}_4$  concentration was 0.0, 0.5, 1.0, or 1.5  $\text{U}_{\text{PO}_4}$ . There were ten cultures for each of these four media. A third series had the same four  $\text{PO}_4$  concentrations, but each of these was tested with eight different pH values, secured by means of small additions of  $\text{HCl}$  or  $\text{NaOH}$ . There were three cultures for each of these 32 media, or 96 in all. These experiments showed no pronounced differences in growth, excepting as the  $\text{PO}_4$  content of the medium influenced the final H-ion equilibrium, which has been considered in an earlier paper (10).

When the  $\text{SO}_4$  of the standard medium was partly or wholly replaced by  $\text{Cl}$ , and also when the concentration of  $\text{SO}_4$  was 1.5  $\text{U}_{\text{SO}_4}$  ( $\text{Na}_2\text{SO}_4$  being added to the medium in the latter case), growth was sensibly the same as in the unmodified standard medium.

When  $\text{CO}_2$  was omitted from the standard medium the resulting modified medium gave greater growth than was obtained with the standard medium itself, and media with  $\text{CO}_2$  concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5  $\text{U}_{\text{CO}_2}$  gave progressively less growth as the  $\text{CO}_2$  concentration was greater. These statements are based on two special experiments with temperature between  $22^\circ$  and  $27^\circ$ , one with weak continuous artificial light and the other with

TABLE IV

	WITHOUT $\text{CO}_2$	WITH $\text{CO}_2$ (MG.-ION PER LITER)				
		0.0075	0.0150*	0.0225	0.0295	0.0370
Average indices (mm.) . . .	99.3	88.9	67.8*	52.1	43.0	35.7
Relative indices . . .	1.47	1.31	1.00*	0.77	0.63	0.53
Final pH values . . .	5.05	5.00	5.10*	5.15	5.30	5.40

\* Standard medium.

stronger continuous artificial light. The actual and relative growth indices derived from the first of these special experiments are shown in table IV, those for the standard medium being marked with asterisks.

Three representative root-tips from this experiment are shown in figure 7. Those grown in the medium without  $\text{CO}_2$  appeared to be in excellent

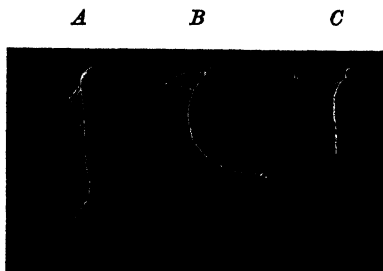


FIG. 7. Representative root-tips grown 2 weeks in media that differed only with respect to the concentration of  $\text{CO}_2$ : A, without  $\text{CO}_2$ ; B and C, with 0.25 mg.-ion per liter (standard) and 0.50 mg.-ion per liter respectively. About natural size.

condition, with an average of eight branches each. Those grown in the standard medium (with 0.015 mg.-ion of  $\text{CO}_2$  per liter) had on the average only five branches each, and those grown in the medium with  $\text{CO}_2$  concentration of 2.0  $\text{U}_{\text{CO}_2}$  were short and stubby and had an average of only two branches.

A repetition of this experiment gave essentially the same results. These series thus support the conclusions indicated by previous experiments with  $\text{CO}_2$ ; namely, that the presence of  $\text{CO}_2$  in the medium tended to have a retarding effect on the development of root-tips grown therein. But it is to be noted that the culture series represented by figure 4 indicated considerably less growth (relative index value of 0.82) when  $\text{CO}_2$  was omitted than was given by the unmodified standard medium.

This retarding influence of  $\text{CO}_2$  as indicated by these experiments, can hardly be attributed directly to low H-ion concentration, for the final pH values were found to be nearly the same whether  $\text{CO}_2$  was present or not, and whether its concentration was high or low, as is seen in table IV. It is perhaps possible that the retarding influence may have been related to Fe supply, as suggested by USPENSKI (8); but it is surprising that these root-tips should have been injured or retarded in their growth by such low concentrations of  $\text{CO}_2$ , an ion that must be generally present at considerable concentrations in rapidly growing tissues in which photosynthesis is not active.

### Summary

1. In a preliminary study reported previously (10), it was found that 3-mm. root-tips excised from wheat seedlings grew excellently for a 2-week

period and without renewal of the liquid culture medium when the inorganic salt contents of the latter were like those specified for the USPENSKI solution (8), and when each liter of medium contained also 20 gm. of dextrose and extract from 0.1 gm. of dry brewers' yeast. Within the limits of that study, the medium thus characterized gave best growth under the following conditions: (1) when its pH value was about 5.0–5.5; (2) when each culture flask, with its single root-tip, contained not less than 25 ml. of medium; (3) when the culture temperature was maintained about 26°–27°; and (4) when the cultures received continuous electric illumination from Mazda "day-light" bulbs. In the present paper are presented results from additional preliminary experiments with root-tips like those used before, in which the experimental variables were the ion concentrations of the culture medium, aside from H-ion and OH-ion concentrations. The medium just described was taken as standard, being used for control cultures, and numerous modifications of it (with respect to inorganic ion concentrations) were tested, generally under the conditions just specified for pH value, volume of medium, temperature, and illumination. The main results may be summarized as follows.

2. The standard medium was notably improved by lowering its Ca concentration by 50 per cent., but its effectiveness was lowered by raising the Ca concentration or by omitting Ca. Raising or lowering the K concentration or omitting K gave poorer growth than was given by the unmodified standard medium. Of 16 systematically chosen combinations of Ca concentration and K concentration (including the omission of one or both of these ions as well as the raising or lowering of their concentrations), only one combination (lowering the Ca concentration by half and keeping the K concentration unmodified) was superior to the one specified for the standard medium, and a single other combination (lowering the Ca concentration and raising the K concentration, each by 50 per cent.) gave an average growth value equaling but not exceeding the corresponding value given by the unmodified standard medium. The remaining tested combinations of these two concentrations were all inferior to the standard.

3. The standard medium was somewhat improved by the omission of Mg and its effectiveness was lowered by raising the Mg concentration. But when  $\text{CO}_2$  was omitted and the Mg concentration was raised by 50 per cent., the effectiveness of the resulting modified medium equaled or slightly exceeded the effectiveness of the unmodified standard medium. In one test, simply omitting  $\text{CO}_2$  led to somewhat poorer growth than was given by the standard medium; but in two special experiments on the influence of  $\text{CO}_2$  concentration, this omission led to very marked improvement of the medium.

4. The standard medium was markedly improved by raising the concentration of  $\text{Fe}_2(\text{SO}_4)_3$  by 50 per cent., and its effectiveness was lowered

by the omission of that salt or by lowering its concentration. Ferric chloride and "ferric citrate" were each found to be unsatisfactory as sources of Fe in these experiments.

5. Omitting  $\text{NO}_3$  or raising or lowering its concentration in the medium exerted no significant effect on growth, and the use of  $\text{NH}_4$  instead of  $\text{NO}_3$ , at various concentrations, was also without considerable influence; it made no difference whether  $\text{NO}_3$  or  $\text{NH}_4$  was used as nitrogen source, or whether either of these ions was at low or at high concentration. It is surprising that excellent growth was obtained in these 2-week cultures, without any nitrogenous substance in the medium excepting such very low concentrations as may have been due to impurities in the salts and dextrose used or in the yeast extract, which was present in all media.

6. Omitting  $\text{SO}_4$  or raising or lowering its concentration in the medium did not show any influence on growth, and the same is true for  $\text{PO}_4$ , excepting as  $\text{PO}_4$  concentration affected the pH value of the medium.

7. The standard medium was improved by the omission of  $\text{CO}_3$ , or at least the effectiveness of the medium was not greatly reduced by that omission. Raising the  $\text{CO}_3$  concentration lowered the effectiveness of the medium.

8. Addition to the standard medium of a considerable concentration of Cl or Na was without notable effect on growth; one or the other of these ions, neither of which was used in the standard medium, was added in the preparation of some of the modified media discussed.

These experiments were carried out at the Boyce Thompson Institute for Plant Research while the writer held a National Research Fellowship in Botany. This account has been prepared for publication at the Laboratory of Plant Physiology of the Johns Hopkins University, with critical help from Professor BURTON E. LIVINGSTON.

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# ACIDITY AND ALKALINITY PRODUCED BY CHANGES IN THE NITROGEN, SULPHUR, AND CARBON CYCLES<sup>1</sup>

JOHN P. CONRAD

(WITH FOUR FIGURES)

## Introduction

Culture media, whether they be soils, nutrient solutions for higher plants, or bacteriological cultures, are subject to fluctuations, often of considerable magnitude, in their H-ion concentrations. Plant and animal fluids and contents of living cells also fluctuate in reaction. One phase of the problem upon which the writer is working involves changes in the reaction of soils, apparently due in part to the changes in the nitrogen cycle. It seemed desirable to investigate in a theoretical way the principal changes that could arise from these and other transformations. The results secured as well as the methods used offer the possibility of wider application than to this specific problem, being perhaps most applicable to the fields of plant physiology, soil chemistry, and bacteriological chemistry. For that reason, they have been prepared for publication.

Certain changes in the nitrogen cycle produce acidity, other changes produce alkalinity, and still other changes in the nitrogen cycle have little or no effect on the pH of the medium. Many of these changes have been worked out quantitatively, others only qualitatively.<sup>2</sup> Confusion exists in some plant and soil literature bearing on these changes. This paper is an attempt to develop by theoretical reasoning, from the fields of inorganic and biological chemistry, a quantitative expression of the acidity and alkalinity produced by changes in the nitrogen cycle, culminating in a chart which shows these relations graphically. Some attention will be given also to production of acidity and alkalinity resulting from transformations within the carbon and sulphur cycles. A subsequent paper will give experimental data bearing on a part of this cycle.

It will be convenient in the following development to speak of the production of one equivalent of "acidity" as meaning also the disappearance of one equivalent of alkalinity, or a fraction of each, such that the sum of the fractional equivalents of acid produced and alkalinity disappearing will equal one; and conversely, with the production of one equivalent of alkalinity.

<sup>1</sup> Contribution from the Division of Agronomy, University of California, Davis, California.

<sup>2</sup> Citations to much of the literature bearing on this point will be reserved for later publication.

As acidity and alkalinity are measured by titrations, it is necessary to have a datum or end-point for reference. I have selected as the reference in the first part of this theoretical development a pH of 4.3, but shall extend the range both to the acid and considerably to the alkaline side of this point. This point was chosen because it represents the pH of the solution used as reference in the methyl orange titrations later employed, and is not far also from the iso-electric point of many of the proteins. The  $\text{CO}_2$  of the atmosphere does not influence titrations at this pH.

#### The cycle: Elementary nitrogen, ammonia, and nitric acid

Since many factors, such as the other chemicals used in the media, the products of other reactions, the incompleteness of the chemical change involving nitrogen, etc., may somewhat complicate the titrations of the original materials and the final end-products of the reaction, it will be simpler to take transformations from the realm of inorganic chemistry in so far as possible. For purposes of comparison, an analogy between heat and acidity will be used. Changes in the amount of calorific heat in a system may arise from at least three different factors:

- A. By addition or withdrawal of heat from the system, *i.e.*, by changing the temperature but not the state of the system.
- B. By physical transformations within the system (such as liquid to vapor, etc.).
- C. By chemical transformations within the system itself.

Similarly, titrable acidity (positive or negative) may change in a system:

- A. By addition of acid to or its withdrawal from the system.
- B. By physical transformations within the system.
- C. By chemical transformations within the system.

The first is easy to understand and the second may occur, but probably not so prevalently with acidity as with heat. Considering factors *A* and *B* as constant in the discussion following, the effect of chemical transformations will be considered; as, for instance, a chemical reaction of great industrial importance:  $\text{S} + \frac{3}{2}\text{O}_2 + \text{H}_2\text{O} = \text{H}_2\text{SO}_4 + 103,240 \text{ calories.}^3$

The heat of this reaction is invariable no matter in how complex a system it may take place, as long as the initial and the final state points are the same. If in a complex system a different heat of reaction is obtained experimentally, this is evidence that some other reaction or reactions have taken place. Likewise if the reactants for this equation could be titrated to an end-point at pH 4.3, and the end-products titrated to the same pH, a change

<sup>3</sup> By computation from heats of formation of  $\text{H}_2\text{O}$  and  $\text{H}_2\text{SO}_4$  given by HODGMAN and LANGE (7).

of two equivalents of titrable acidity for each gram-atom of sulphur transformed will be secured. This reaction will always give these results no matter in how complex a system it takes place, provided the initial and final state points are the same and the end-point of titration is above pH 4. This must be true or the law of the conservation of matter would be violated.

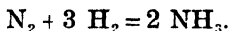
As with the heat of reaction so also with the titrable acidity of this reaction, it is immaterial whether the reaction occurs all at once or by stages.

If the sulphur is used up in this reaction and it is known to be oxidized according to the reaction given, the change resulting in the increase of sulphate-ion is directly proportional to the heat evolved and also to the change in titrable acidity resulting from *this reaction*. Since in this development physical conditions are considered as being kept constant, and no additions of heat or titrable acidity are being made, no heat can be evolved nor titrable acidity arise except through chemical transformations.

From the preceding analogy, it follows that changes in the amount of titrable acidity (considering alkalinity as negative acidity) are equal to the algebraic sum of the titrable acidities arising from each of the reactions taking place. In other words, in any system the total changes in titrable acidity (factors *A* and *B* remaining constant) are equal to the algebraic sum of the changes due to each individual reaction. It is the titrable acidity arising from each of many of the possible reactions within the nitrogen, sulphur, and carbon cycles which will be considered.

#### ELEMENTARY NITROGEN TO AMMONIA

A chemical process recently coming into great commercial prominence is the Haber-Bosch method for making gaseous ammonia. The reaction generally ascribed to this process is as follows:

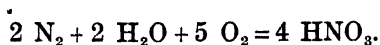


Since the reactants are not titrable with acid or alkali and therefore have a zero titration value, and since the  $\text{NH}_3$  if dissolved in water may be titrated, requiring 1 mol of acid for each mol of  $\text{NH}_3$ , then for each gram-atom of nitrogen fixed as ammonia one<sup>4</sup> equivalent of base (in this case ammonia) is produced. Thus a change of one equivalent of titrable alkalinity is produced for each gram-atom of nitrogen transformed from  $\text{N}_2$  to  $\text{NH}_3$ , no matter whether the action goes on in the Haber-Bosch process, or in nature through the fixation of nitrogen by a legume and the subsequent decay of this crop's residues to form  $\text{NH}_3$ .

#### ELEMENTARY NITROGEN TO NITRIC ACID

In the arc method of fixing gaseous nitrogen the process takes place in several steps, but a chemical reaction which would express the original materials and the final product is as follows:

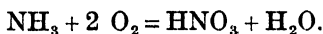
<sup>4</sup> Theoretically it may be slightly less than one, as  $\text{NH}_3$  is a weak base.



The initial material is considered to have zero titration value, but with the end-product of the process, nitric acid, one equivalent of acid is produced for each gram-atom of nitrogen changed.

#### AMMONIA TO NITRIC ACID

The transformation of ammonia to nitrate in the soil now has a counterpart in industry known as the Ostwald process (11). By the use of a platinum catalyst and in several steps, the ammonia gas is transformed to nitric acid by the following over-all reaction:

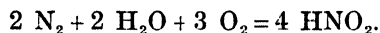


On titration, the chemicals on the left side of the equation give one equivalent of base, and those on the right one equivalent of acid, for each gram-atom of nitrogen transformed. When nitrogen is changed from ammonia to nitric acid there is a net change of titrable acidity equal to two equivalents of acid for each gram-atom of nitrogen transformed.

If  $\text{NH}_3$  is neutralized by sulphuric acid to form  $(\text{NH}_4)_2\text{SO}_4$  and the nitrogen in the ammonium salt oxidized to nitrate, there will be present the original sulphuric acid and the nitric acid formed. In this transformation for each mol of ammonium sulphate there will be produced four equivalents of acid. As there are two gram-atoms of nitrogen per mol of ammonium sulphate, there is an increase of two equivalents of titrable acidity for each gram-atom of nitrogen transformed.

#### ELEMENTARY NITROGEN TO NITROUS ACID

When the gases in the arc process are dissolved in water, nitrous acid is formed according to the reaction:



Thus for each gram-atom of nitrogen transformed from elementary nitrogen to nitrous acid, one equivalent of titrable acidity is produced except as will be brought out later.

#### AMMONIA TO UREA

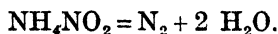
If ammonium carbonate is heated under appropriate conditions, urea results as follows:



Titration of the initial  $(\text{NH}_4)_2\text{CO}_3$  we get an equivalent of alkalinity for each gram-atom of nitrogen, while practically urea, because it is a very weak base, gives none, except as will be brought out later when the acidity of the system is high.

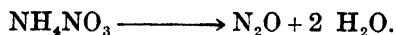
### Other nitrogen transformations

From the preceding, we would predict that the transformation of nitrates back to  $N_2$  would produce titrable alkalinity, and that of  $NH_3$  to  $N_2$  produce titrable acidity. As these reactions do not normally occur in inorganic chemistry, some reactions involving the formation of  $N_2$  from nitrogen salts may be investigated. If the alkalinity produced by the formation of  $NH_3$  from the union of  $N_2$  and  $H_2$  is neutralized by nitrous acid, ammonium nitrate is produced. On titrating, this will be practically neutral. But ammonium nitrite decomposes on heating to form elementary nitrogen and water as:



The final products are not titrable with acid or alkali and hence have a zero titration value. Very little or no change of titrable acidity is brought about by this reaction.

On the other hand, if the alkalinity of ammonia is neutralized with nitric acid, ammonium nitrate is formed, which has for many practical purposes no titrable acidity. But  $NH_4NO_3$  decomposes on heating to give nitrous oxide ( $N_2O$ ) and water, according to the following reaction:



If nitrous oxide is dissolved in water, a very weak acid, weaker than carbonic, is formed,  $H_2N_2O_2$ . This acid is called hyponitrous acid. Only at high pH values would its acidity become manifest. Figure 1 illustrates graphically these relationships.

Except as will be brought out later,  $N_2$ , urea, ammonium nitrite, and ammonium nitrate are for most practical purposes neutral in reaction.

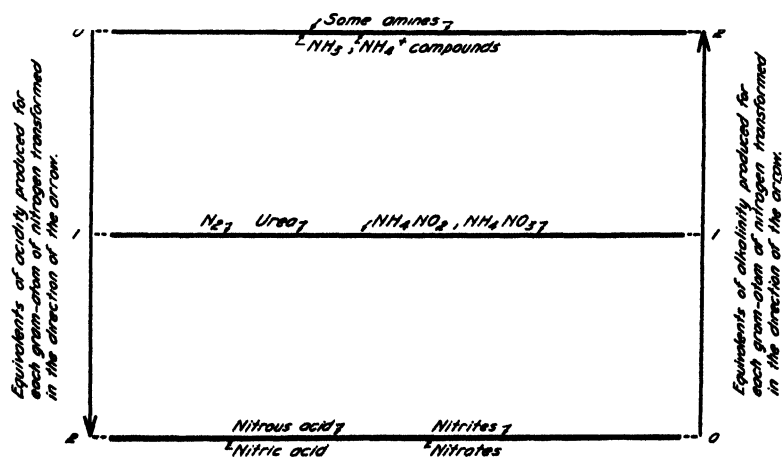


FIG. 1. Titrable acidity and alkalinity produced by changes in nitrogen cycle. Very little error arises in using this chart between pH values of 4.5 and 8 (see fig. 2).

Transformation of one gram-atom of nitrogen from any or all of the preceding substances to ammonia will result in the production of one equivalent of alkalinity. Likewise the transformation of one gram-atom of nitrogen from this group of substances to nitrate or nitric acid will result in the production of one equivalent of titrable acidity, no matter in how complex a system it takes place nor in how many stages, subject of course to the condition that factors *A* and *B* remain constant.

### Transformations of protein nitrogen

From solution-culture and field studies, some details of which will be taken up in a subsequent paper, it is apparent that the medium becomes more acid when ammonium salts of strong acids, with the exception of  $\text{NH}_4\text{NO}_3$ , are used as a plant nutrient; also the medium becomes more alkaline when nitrates, except  $\text{NH}_4\text{NO}_3$ , are used. On the average, therefore, the compounds of nitrogen in the plant must have a position on the chart somewhere intermediate between  $\text{NH}_3$  on the one hand and  $\text{HNO}_3$  on the other. Proteins are undoubtedly the chief class of nitrogenous compounds in normal plants, and therefore could be expected to lie on the chart somewhere between ammonium compounds and nitrates.

A more direct means of determining the position of a given protein lies (1) in a consideration of its titration curve, and (2) in a consideration of its iso-electric point in so far as that might represent the pH of a suspension of the pure protein in water. This assumption is on the basis of LOEB's work (8, p. 35). In soils, MATTSON (9) has shown that when the iso-electric point is not at neutrality, some differences may arise between the two values.

A few proteins will be considered as typical of all proteins. Discrepancies arising because any protein does not behave as do the types are, it is believed, of relatively minor importance in this connection. Some of the proteins selected are not found in plants, but because the titration curve and elementary composition of each are known, they are included. Those chosen, and the properties of each applicable to the present problem, are given below:

Protein	Chemical elements present	pH of aqueous suspension	Investigators cited
Gelatin	C, H, O, N	4.7	LOEB (8, figs. 7, 13)
Durumin	C, H, O, N, S	5.9	HOFFMAN and GORTNER (6, table 78)
Teozein	C, H, O, N, S	5.2	" (6, table 79)
Fibrin	C, H, O, N, S	4.9	" (6, table 81)
Egg albumin	C, H, O, N, S, P	4.8	LOEB (8, figs. 4, 11)
Casein	C, H, O, N, S, P	4.7	LOEB (8), HOFFMAN and GORTNER (6, table 80)

If we completely oxidize each of these proteins in turn with oxygen, dissolve the end-products of the reaction in water, and titrate them to our reference pH of 4.3, in equilibrium with the atmosphere, the following results are secured:

In the original protein each gram-atom of	produces one mol each of the acid below	which titrated at pH 4.3 gives the number of equivalent of acidity below	Remarks
C	H <sub>2</sub> CO <sub>3</sub>	0	lost to the air
N	HNO <sub>3</sub>	1	almost fully neutralized
S	H <sub>2</sub> SO <sub>4</sub>	2	"
P	H <sub>3</sub> PO <sub>4</sub>	1	only 1/3 neutralized

The nitrogen transformation from the protein, gelatin, at its iso-electric point to HNO<sub>3</sub> titrated at pH 4.3 would produce one equivalent of acidity. Gelatin may then be placed tentatively with N<sub>2</sub> on the chart. But it required (8, fig. 7) about 1.5 cc. of 0.1 N HCl or H<sub>2</sub>SO<sub>4</sub> to bring 1 gm. of purified gelatin in 100 cc. of H<sub>2</sub>O from its iso-electric point of pH 4.7 to 4.3, our reference point in titration. One gm. of gelatin assumed to contain 17.80 per cent. nitrogen fully oxidized would give 127 cc. of 0.1 N HNO<sub>3</sub>. The error then in placing gelatin with N<sub>2</sub> on the chart at pH 4.3 would be approximately 1.5 divided by 127, or 1.18 per cent. Considering the HNO<sub>3</sub> formed as a measure of the nitrogen transformation alone (the H<sub>2</sub>SO<sub>4</sub> and H<sub>3</sub>PO<sub>4</sub> formed being credited to sulphur and phosphorus transformations) the errors involved in placing each of the proteins mentioned at N<sub>2</sub> on the chart can be computed in a similar manner. These are for durumin about 0.7 per cent.; for teozein, 0.1 per cent.; for fibrin, 0.3 per cent.; for egg albumin, 0.8 per cent.; and for casein, 0.5 per cent.

It would be desirable to know the errors involved at other pH reference points. If the titration end-point be pushed up to the alkaline side soon a place is reached where no end-point can be secured in equilibrium with the atmosphere because of the CO<sub>2</sub>. We can then assume titration of the original gelatin and the products of its complete oxidation in a CO<sub>2</sub>-free atmosphere. Under such conditions, the CO<sub>2</sub> produced by the oxidation would be eliminated and the difference would be due to the nitrogen change alone.

In titrating 0.8 gm. of purified gelatin in 100 cc. at a pH of 8.4 (about the phenolphthalein end-point), nearly 2.9 cc. of 0.1 N NaOH is required (8, fig. 13). The same amount of gelatin fully oxidized in equilibrium with a CO<sub>2</sub>-free atmosphere would require 101.5 cc. of the same strength of NaOH to neutralize it. At pH 8.4, then, an error of about 2.9 per cent. results from placing gelatin at the position of N<sub>2</sub> on the chart.

By arbitrarily attributing all of the error involved in the titration curve of each protein as due to the nitrogen change alone, the errors involved in placing these proteins at pH 8.4 at the position of  $N_2$  on the chart are for durumin, 0.4 per cent.; for teozein, 0.4 per cent.; for fibrin, 4.0 per cent.; for egg albumin, 3.3 per cent.; and for casein, 7.0 per cent.

Assuming no error from the titration curve as due to sulphur and phosphorus changes, each gram-atom of sulphur in the protein would result in practically two equivalents of titrable acidity at pH 8.4 due to complete oxidation of the protein, while each gram-atom of phosphorus would likewise result in about two equivalents of titrable acidity ( $H_3PO_4$  is about  $2/3$  neutralized at pH 8.4).

With the exception of gelatin, each of the proteins being considered results, with complete oxidation, in acidity in excess of that due to the nitrogen change. Acidity arising from the  $H_2SO_4$  and  $H_3PO_4$  must be taken into consideration.

With the definite placing of proteins on the nitrogen chart it is clearly seen that the transformation from nitrates to proteins will result approximately in one equivalent of titrable alkalinity for each gram-atom of nitrogen changed. At the same time additional alkalinity will result from the sulphur transformation from sulphate to protein. Though phosphorus is normally not a constituent of plant proteins, it may enter into compounds whose titration curves are similar. Additional alkalinity would then result. Any deviation from this would be contrary to the law of the conservation of matter, and therefore invalid.

Although it would be desirable to have the pH of a suspension or solution of the pure protein in pure water as the best experimental evidence at hand, such a pH may be secured indirectly by assuming that such a suspension is at, or very near, the iso-electric point of the protein in question. Assuming again that other methods of securing the iso-electric point will give indirectly the pH of a water suspension of the pure protein, the iso-electric point of some other proteins is of interest.

Thus CSONKA, MURPHY, and JONES (5) report iso-electric points of five albumins from pH 4.2 to 5.5, thirty globulins from pH 4.5 to 5.5, and five prolamines from pH 5.9 to 6.6. CSONKA and JONES (4) report two glutelins of wheat and one each of rice, oats, and corn as having iso-electric points of pH 6.45. PEARSALL and EWING (10) report the iso-electric points of some plant proteins as found by the precipitation methods. These varied from pH 3.2 to 5.6. Excluding three extremes, the rest varied but little from pH 4.5. CHIBNALL (3) reports iso-electric points of leaf-cytoplasmic proteins from pH 3.5 for rhubarb to pH 5.1 for broad bean, with an average near pH 4.4. Other iso-electric points have been determined by others, but

these in general fall within the range already given, with the possible exception of glutenin with an iso-electric point of 7.0, reported by TAGUE (13).

It is evident that many if not most of the proteins have iso-electric points close to those here presented whose titration curves are available. These observations allow us then to place proteins at the same place on the chart as  $N_2$ , considering the errors presented almost negligible for many purposes.

Table I lists some of the nitrogen transformations and the titrable acidity and alkalinity resulting from each at usual pH values.

TABLE I

NITROGEN TRANSFORMATIONS	NET PRODUCTION IN EQUIVALENCES OF ACIDITY AND ALKALINITY PER GRAM-ATOM OF NITROGEN TRANSFORMED	
	ACIDITY	ALKALINITY
Nitrogen fixation by legumes and bacteria to form proteins	0	0
Urea to proteins; proteins to urea	0	0
Assimilation of $NH_3$ by plants (including lower forms) to form proteins	1	-1
Nitrification of urea, proteins, etc. (net effect)	1	-1
Nitrification of $NH_3$ and $NH_4$ compounds	2	-2
Assimilation of nitrates ( $HNO_3$ ) by plants to form proteins	-1	1
Denitrification (nitrates to $N_2$ )	-1	1
Reduction of nitrates to ammonium compounds (14, p. 182)	-2	2

Some of the work done on denitrification is of interest in connection with table I. Alkalinity is produced when nitrates are denitrified and elementary nitrogen is produced. BURRI and STUTZER (2), in their work on denitrifying bacteria, found that the cultures rapidly developed alkalinity as the fermentation progressed. Although no figures are given, they found higher alkalinities to develop than could be attributed to decomposition of the nitrates. This increase they attributed to the transformation of some of the alkali citrates used in the media to alkali carbonates, and to the utilization of some more of the citrate radicle as a source of carbon by the organisms.

By causing denitrification to take place with  $NaNO_3$ , and especially with  $Sr(NO_3)_2$ , SACHAROWA (12) has carried the pH in one step to 8.5 and in another step from 8.5 to 9.4. No figures are available so far as we know to determine experimentally a quantitative relationship between the alka-

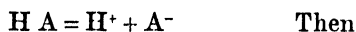
linity produced and the nitrogen transformed. According to our chart, for each gram-atom of elementary nitrogen formed there should be one equivalent of alkalinity produced.

An experiment performed by BRIOUX (1) is also of interest in the light of the relations shown in figure 1. Using 0.5 gm. of urea in 500 gm. of soil having an initial pH of 6.45, he secured a pH of 8.5 in 48 hours, with a heavy production of ammonia as shown by Nessler's reagent; and after 76 days the pH had changed to 5.35 with the transformation of ammonia to nitrate. Our chart would have qualitatively predicted these results.

### Weak nitrogenous acids and bases

Nitrogen transformations given in figure 1 that take place between pH 5 and pH 8 follow the chart very closely and hence undoubtedly apply to most culture media. On account of the hydrolysis of weak acids and bases, however, errors of considerable magnitude may creep in where the pH is beyond these limits.

Let us consider the dissociation of the weak acid H A.



$$\frac{[\text{H}^+][\text{A}^-]}{[\text{H A}]} = K_a$$

$$\log [\text{H}^+] + \log \frac{[\text{A}^-]}{[\text{H A}]} = \log K_a \quad \text{But } \log [\text{H}^+] = -\text{pH}$$

$$\log \frac{[\text{A}^-]}{[\text{H A}]} = \log K_a + \text{pH} \quad \dots \quad (1)$$

In the case of a definite weak acid,  $K_a$  is known. Then for a definite pH the ratio  $[\text{A}^-]$  to  $[\text{H A}]$  is fixed and can be calculated. If the system be analyzed for  $[\text{A}^-]$  the undissociated  $[\text{H A}]$  is secured also. With the sum of  $[\text{A}^-] + [\text{H A}]$  known and their ratio  $[\text{A}^-]/[\text{H A}]$  calculable, the concentration of  $\text{A}^-$  may be computed.<sup>5</sup> With a transformation in which H A is formed from some other substance (as for example  $\text{HNO}_2$  from  $\text{N}_2$  in figure 1), the change in the number of equivalents of  $\text{H}^+$  which resulted from the transformation is equal to the change in the number of equivalents of  $\text{A}^-$  produced by that transformation. The transformation affects the titrable acidity and in any but a highly buffered system this will change the pH, sometimes materially. The amount of such change in pH is dependent upon the buffer characteristics of the particular system. A transformation

<sup>5</sup> Other undissociated molecules or complex ions in equilibrium with  $\text{A}^-$  would lead to error by the use of this procedure, although the general theory is not invalidated thereby.

which would cause a great change in pH in a poorly buffered system would cause a very much smaller change in pH in a highly buffered system. Further consideration of this phase of the problem is beyond the scope of this paper. It is obvious that only the  $H^+$  dissociated from the total  $HA$  formed is effective upon the pH of the system or in titration. The fraction of the total  $HA$  dissociated is

$$\frac{[A^-]}{[HA] + [A^-]} = \frac{\frac{[A^-]}{[HA]}}{\frac{[HA] + [A^-]}{[HA]}} = \frac{\frac{[A^-]}{[HA]}}{1 + \frac{[A^-]}{[HA]}},$$

i.e., the fraction dissociated is equal to the ratio  $\frac{[A^-]}{[HA]}$  divided by that ratio plus one.

For  $HNO_2$  we have by substitution in equation (1)

$$\log \frac{[NO_2^-]}{[HNO_2]} = \log (4 \times 10^{-4}) + pH.$$

In table II are given the values of the ratio  $\frac{[NO_2^-]}{[HNO_2]}$  and the decimal fraction dissociated for given values of pH.

TABLE II

CALCULATION OF THE FRACTION OF  $HNO_2$  DISSOCIATED AT DIFFERENT pH VALUES; DISSOCIATION CONSTANT OF  $HNO_2 = K_a = 4 \times 10^{-4}$

pH	$\log \frac{[NO_2^-]}{[HNO_2]}$	$\frac{[NO_2^-]}{[HNO_2]}$	FRACTION DISSOCIATED
6.0	2.6	400	0.997
5.0	1.6	40	0.975
4.0	0.6	4	0.80
3.5	0.1	1.26	0.557
3.4	0.0	1.0	0.50
3.0	-1.6	0.4	0.286
2.0	-2.6	0.04	0.038
1.0	-3.6	0.004	0.004

Thus at pH 3.4 each gram-atom of nitrogen transformed to  $HNO_2$  will result in but one-half of an equivalent of titrable acidity, and at pH 2.0

only 0.038 equivalent of titrable acidity, while at pH 4.0 there will be 0.80 equivalent.

A weak base dissociates into  $\text{BOH} \rightleftharpoons \text{B}^+ + \text{OH}^-$  and  $\frac{[\text{B}^+][\text{OH}^-]}{[\text{BOH}]} = K_b$

$$\log [\text{OH}^-] + \log \frac{[\text{B}^+]}{[\text{BOH}]} = \log K_b$$

But  $\log [\text{OH}^-] = \text{pH} - 14$  (approximately at  $25^\circ\text{C}.$ )

$$\text{Then } \log \frac{[\text{B}^+]}{[\text{BOH}]} = \log K_b + 14 - \text{pH}.$$

The dissociation constant of  $\text{NH}_4\text{OH}$  is  $1.8 \times 10^{-5}$ , and of urea is  $1.5 \times 10^{-14}$ . These data permit the setting up of similar tables for the fractions dissociated at different pH values for these substances (fig. 2). A typical protein, durumim, is also included from the titration curves mentioned earlier (from data of HOFFMAN and GORTNER).

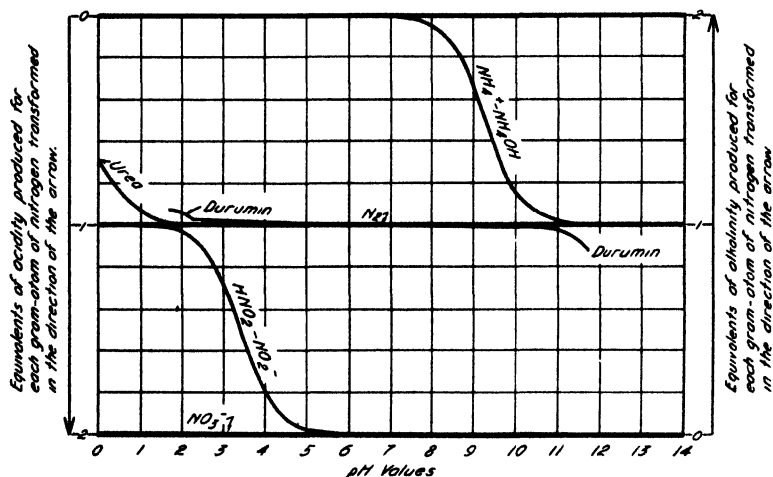


FIG. 2. Titrable acidity and alkalinity produced by changes in nitrogen cycle at different pH values. Proteins represented by durumim, the curve of which is plotted from data of HOFFMAN and GORTNER (6); other proteins have different curves.

When weak acids and bases are involved in these charts, the pH of the system is of very great importance in determining the magnitude of the changes in acidity. In a poorly buffered system the transformations may cause marked changes in pH. For these conditions the chart gives with considerable accuracy the amount of strong acid or base which must be added to keep the system at a constant pH.

It is believed that the number of transformations given here may be extended by the simple expedient of using or finding the titration curves or dissociation constants of the bases and acids not shown. Thus other

proteins, the various amino acids, plant alkaloids, etc., may be added. It is realized that a small source of error may arise because of the use of the concentration formulas rather than of a consideration of the activity of the various ions involved. It is believed, however, that this development together with the charts will give a general picture of the results of these transformations sufficiently accurate for many purposes.

### Sulphur cycle

In biology, transformations of sulphur are also important. As is well known, these may influence the H-ion concentration of the medium. From a consideration of the dissociation constants of the various acids of sulphur, similar tables and a chart may be set up. These acids dissociate in two stages, each with a different constant. The values given by HODGMAN and LANGE (7) are as follows:

ACID	CONSTANTS FOR	
	FIRST HYDROGEN	SECOND HYDROGEN
$\text{H}_2\text{S}$	$9.1 \times 10^{-8}$	$1.2 \times 10^{-15}$
$\text{H}_2\text{SO}_3$	$1.7 \times 10^{-2}$	$5 \times 10^{-6}$
$\text{H}_2\text{SO}_4$	.	$2 \times 10^{-2}$

Figure 3 shows the effect of these transformations on the titrable acidity

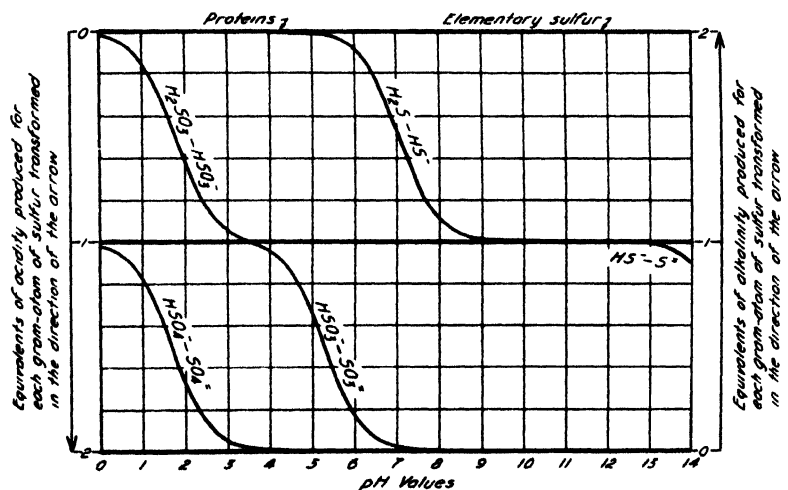


FIG. 3. Titrable acidity and alkalinity produced by changes in sulphur cycle at different pH values.

at various pH values. Since the values for the titration curves of the proteins have been put into the nitrogen chart, the proteins in the sulphur chart are kept at neutrality through all of the pH values. It is interesting to note that a transformation of a gram-atom of sulphur as sulphide to

sulphate at pH 10 gives but one equivalent of titrable acidity, while the same transformation at pH 4 will result in two such equivalents. At pH 10 the sulphides occur as BHS and the transformation is then to  $\text{BHSO}_4$ , which at that pH is fully effective as a mixture of  $\text{B}_2\text{SO}_4$  and  $\text{H}_2\text{SO}_4$ ; while at pH 4 the sulphide ion occurs practically undissociated as  $\text{H}_2\text{S}$  and this is transformed to  $\text{H}_2\text{SO}_4$ , which at that pH is practically completely effective as a fully dissociated acid.

### Carbon cycle

As is well known, transformations within the carbon cycle influence the pH of the culture medium. The dissociation constants of many organic acids are available, but only a few will be considered here in order to reduce the confusion of the chart to a minimum. The dissociation constants of these from HODGMAN and LANGE are as follows:

ACID	DISSOCIATION CONSTANTS FOR	
	FIRST HYDROGEN	SECOND HYDROGEN
$\text{H}_2\text{CO}_3$	$3 \times 10^{-7}$	$6 \times 10^{-11}$
Acetic $\text{CH}_3\text{COOH}$	$1.86 \times 10^{-5}$	
Formic $\text{HCOOH}$	$2.14 \times 10^{-4}$	

As compounds of carbon, the sugars, alcohols, and proteins are considered as not contributing to the titrable acidity or alkalinity. It is true that the proteins have a titration curve, but these contributions of the proteins to titrable acidity are for convenience attributed to the nitrogen cycle. Figure 4 brings out some of the relationships in the carbon cycle.

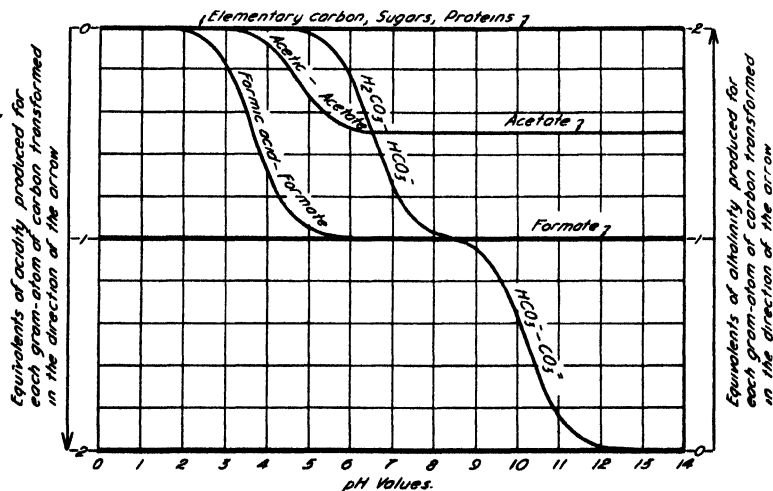


FIG. 4. Titrable acidity and alkalinity produced by changes in carbon cycle at different pH values.

Because of the weak acids involved, some interesting points are brought out. In a highly buffered system, one gram-atom of carbon as sucrose transformed to  $\text{H}_2\text{CO}_3$  at pH 12 or 13 will cause about two equivalents of titrable acidity to appear; at pH 8 to 9, about one equivalent of acidity; and at pH below 4.5, practically no change in titrable acidity.

The change of sugars to acetic acid, which has two gram-atoms of carbon per mol, results in the production of about one-half equivalent of titrable acidity for each gram-atom of carbon transformed in the region from pH 6 to more alkaline reactions. With the oxidation of acetic acid further to  $\text{CO}_2$ , especially at pH 5, the medium becomes more alkaline while at pH 12 it becomes more acid.

The transformations with formic acid are especially interesting. At pH 12 to 13 one gram-atom of carbon as formic acid is transformed into  $\text{H}_2\text{CO}_3$  with the production of about one equivalent of titrable acidity; at pH 8 to 9, with little or no acidity or alkalinity; and at pH 5, with the production of nearly one equivalent (0.9) of titrable alkalinity.

### Summary

1. Relying upon the law of conservation of matter and from simple reactions in the main, it is shown that transformations from one form of nitrogen to another within the group  $\text{N}_2$ , urea,  $\text{NH}_4\text{NO}_3$ ,  $\text{NH}_4\text{NO}_2$ , and proteins cause very little or no change in titrable acidity or alkalinity. Transformations from any one or all of this group to ammonia cause the appearance of about one equivalent of titrable alkalinity for each gram-atom of nitrogen changed. Transformations from this same group to nitric acid or nitrates result in the production of about one equivalent of titrable acidity for each gram-atom of nitrogen changed.

2. When weak acids and bases are formed, the pH of the medium is important in determining the amount of titrable acidity and alkalinity produced. This is especially true in some of the transformations that may take place in the sulphur and carbon cycles. Charts showing these relationships are given.

The writer is indebted to Dr. C. S. BISSEON for many helpful suggestions during the preparation of the manuscript.

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# PHOTODYNAMICALLY INDUCED TROPISMS IN PLANT ROOTS<sup>1</sup>

H. F. BLUM AND K. G. SCOTT

(WITH FOUR FIGURES)

METZNER (7) found that a positive phototropic response could be induced in the roots of wheat seedlings by growing them in dilute solutions of fluorescein dyes. Normally the direction of growth in these roots is not influenced by light, but the presence of a very small concentration of dye (*e.g.*, 1/500,000) brings about a definite bending of the roots toward the light. This phenomenon is of interest from two standpoints: the information it may give as to the mechanism of tropic bending in plants, and the bearing it may have on the phenomenon of photodynamic action in general. In conducting the investigations described in this paper, we have been concerned with both of these questions.

## Method

In most of the experiments the wheat seedlings were grown on cheese-cloth suspended at the surface of a solution contained in a glass vessel. Roots so grown readily penetrate the cheese-cloth mesh and the direction of their growth may be observed through the walls of the vessel. In most experiments a solution was used having the following composition:  $\text{KH}_2\text{PO}_4$ , 0.001 M;  $\text{KNO}_3$ , 0.05 M;  $\text{MgSO}_4$ , 0.002 M;  $\text{CaNO}_3$ , 0.05 M. This solution has a hydrogen-ion concentration corresponding to about pH 4.5. In some experiments the concentration was adjusted to other values by the addition of 0.1 N KOH. To these solutions, erythrosin<sup>2</sup> was added when it was desired to produce phototropic responses.

It was found that the effect of cylindrically walled vessels upon the direction of the light rays did not interfere with the production of phototropism, and various sized vessels of this type were used to suit the convenience of particular experiments. Light sources were several types of clear tungsten-filament lamps. Various factors modify the magnitude of the reaction, of course, such as the concentration of the dye and the thickness of the dye solution between the light source and the roots; in any one set of experiments these factors were maintained as nearly constant as possible. A convenient arrangement for observing the phenomenon was found to be as follows: The roots were suspended at approximately the center of a cylindrical glass vessel 6 cm. in diameter, in a solution of 1/500,000 erythrosin

<sup>1</sup> This research was assisted by a grant from research funds of the University of California.

<sup>2</sup> Erythrosin Schultz no. 912, made by National Aniline Company. This is tetra-iodo-fluorescein.

at about pH 4.5, with a 40-watt concentrated tungsten-filament lamp, 40 cm. distant from the roots. These were approximately the conditions in most of our experiments; ordinarily they were performed in a black-walled room; otherwise precautions were taken to avoid any appreciable amount of light from sources other than the direct radiation from the experimental source.

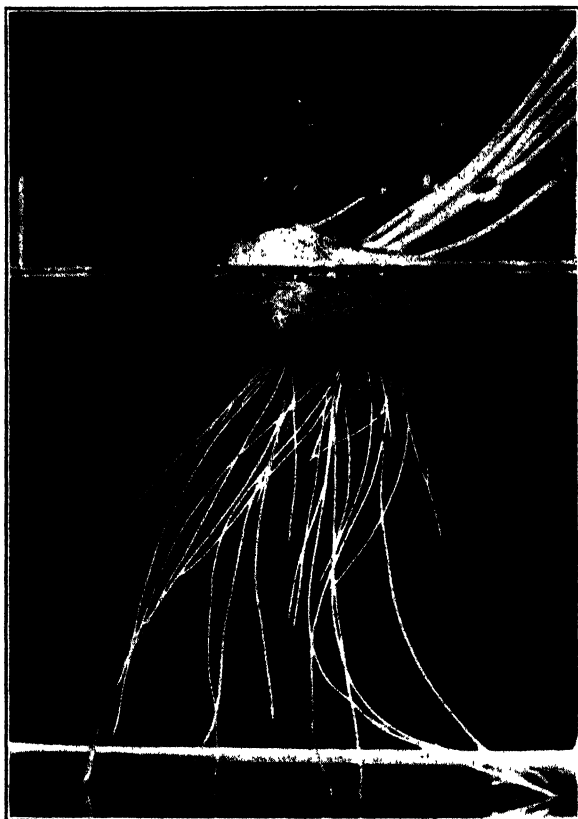


Fig. 1. Normal non-phototropic wheat roots, age 10 days. Light source on the right.

### Description of the phenomenon

Under the conditions just described the roots show a definite orientation toward the light source within the course of a few hours. Usually the orientation appears as an abrupt bending toward the source of light, regardless of the position of the source (compare figure 1<sup>a</sup> with figures 2<sup>a</sup> and 3<sup>a</sup>). In

<sup>a</sup> It was found necessary to transfer the plants to flat-walled vessels for photographing. This disturbed the roots to some extent so that the orientation appears somewhat less exact than in the original vessels.

some cases, by placing the source above the seedlings, it was possible to produce bending of the roots through almost  $180^\circ$ , so that they grew nearly vertically upward instead of in the normal positively geotropic manner. In some cases a curious unoriented bending occurs before orientation toward the light becomes definite (figure 2); this is probably due to the effect of the dark reaction of the dye, which will be discussed in a later section, and possibly also to the shading of some roots by others at certain points in

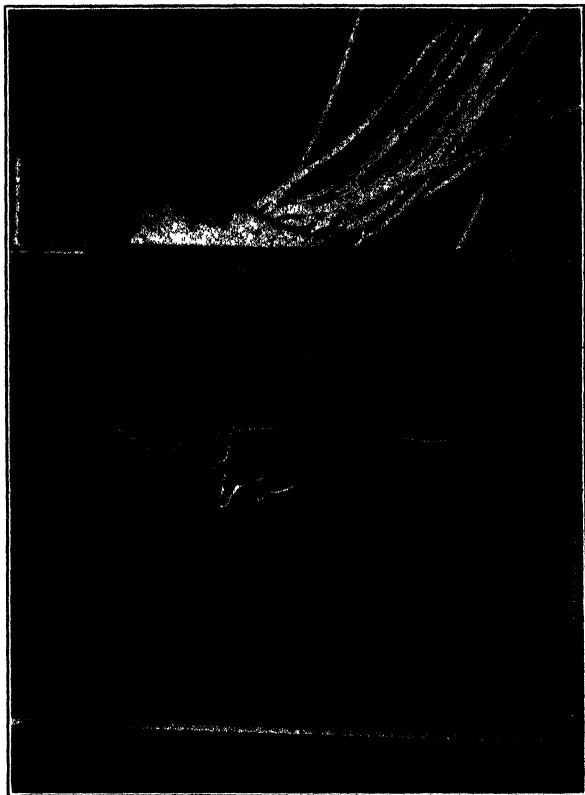


FIG. 2. Wheat roots 10 days old, sensitized after 5 days. Light source on the right.

their bending. The growth of the roots after they have become oriented is usually not in a straight line, but in a series of short waves as is shown in figure 3 at A. It would seem that the root is caused to bend toward the light but bends too far and is reoriented.

METZNER (7) has described variations in appearance of the induced tropism. He has separated the phenomenon into two types on this basis, active bending and passive bending. The former, in which the curvature is gradual, is produced by weak light and low dye concentrations. The

latter, in which the curvature takes place at an acute angle, is produced by stronger light and greater concentrations of the dye. We have been able to produce a great variety of shapes by altering the dye concentration and light intensity, but it seems that METZNER's types simply represent different degrees of the same process, and that there is no real justification for the separation of the two types.

### Importance of the growing tip

It was found that the orientation occurs only in the region of the root tip. If a few millimeters of the tip are cut off, the root no longer orients toward a light source. Likewise, if a beam of light is directed above the region of the tip of a normally downward growing root, no bending occurs. This experiment was performed by restricting the light to a narrow band striking the root well above the tip. No bending of roots so illuminated was observed, but roots developing later in the same culture were definitely oriented as soon as they grew down into the beam of light.

These results offer an explanation for the zigzag growth of the oriented roots described above (figure 3 A). The extreme tip of the root, or root cap, is composed of a large number of cells which proliferate from the root meristem but do not contribute to the elongation of the root; the significant length growth takes place in the region just behind the root cap, as a result of active cell division and increase in cell size in a limited zone. The phototropic orientation is apparently accomplished by a differential growth on opposite sides of this zone of active growth, the growth being less rapid on the side toward the light source, so that the root tip bends in that direction. In paraffin sections this differential growth appears as a less degree of elongation of the cells on the illuminated side of the root than on the dark side. When by this process of differential growth the long axis of the root becomes oriented parallel to the light rays, the apical region of the root shields the actual growing region from the light, so that the effect of the light upon growth ceases. The differential rate of growth continues for a time, however, until the tip reaches a position at which it is differentially illuminated again. The side of the root previously least illuminated now receives the greater amount of light and orientation occurs in the opposite direction. This process is repeated, producing the effect illustrated in figure 3 at A.

Under the microscope we have been unable to determine from paraffin sections whether there is any degree of penetration of the dye into the roots. The macroscopic staining of the roots, however, indicates a considerable accumulation of the dye at the surface at least. This staining is unequal, in general being greatest in the root cap and least in the region of growth just behind. It is probable that the least viable cells are the most readily stained, as is characteristic for fluorescein dyes, and that the staining in the

less rapidly growing regions may be due to the accumulation of the dye in dead or dying cells, particularly in the region of the root cap where cells are being constantly sloughed off under normal conditions. Certainly there is no indication that the macroscopic accumulation of the dye marks the region of greatest effective photochemical action.

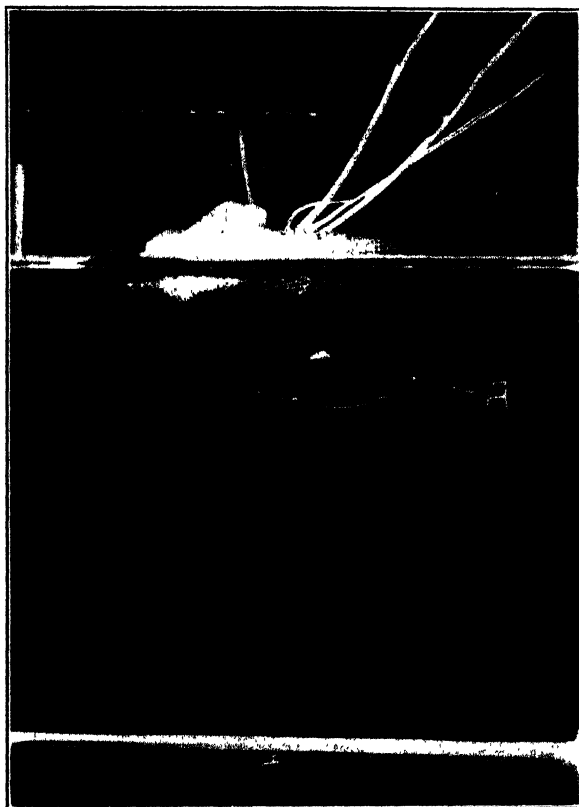


FIG. 3. Wheat roots 10 days old, sensitized after 2 days. Light source on the right.

#### Dark reaction

Most photodynamic dyes produce destructive effects in cells when in sufficient concentration, even when they are carefully protected from light (BLUM 3). This is particularly true of the fluorescein dyes, so that it is not surprising to find a marked effect of non-irradiated erythrosin on wheat roots. BOAS and MERCKENSCHLAGER (4) found that the roots of sprouting barley seeds were caused to undergo strange, unoriented bending by the addition of fluorescein dyes to the solution in which they were sprouted, although they were not actually growing in the solution. Our wheat roots

were likewise found to exhibit unoriented bendings within a few hours after they were introduced into the erythrosin solutions, although carefully maintained in the dark; these would seem to be similar to those described by BOAS and MERCKENSCHLAGER. It was also observed that growth of the roots was distinctly delayed. It seems probable that the bending is due to the unequal rate of penetration of the dye on opposite sides of the root, growth being delayed most on the side where penetration is the most rapid.

BLUM (1) suggested that the photodynamic phenomena as observed are the resultant of two reactions, the dark reaction and the photo-reaction. BLUM and MCBRIDE (2) have also shown that the two reactions are essentially different in that the photo-reaction requires the presence of molecular oxygen, whereas the dark reaction does not. It would seem that in the case of plant roots the dark reaction is capable of producing root bending by the differential delay of root growth, but that this differential effect is increased and given a definite orientation by the light reaction. The roots usually continue in a more nearly straight-line growth after the first bending, when it may be assumed the dye has penetrated more evenly at all surfaces of the root.

Parallel with the findings of JODLBAUER and HAFFNER (6) and of BLUM (1) that the hemolysis of red blood cells by non-irradiated fluorescein dyes is favored by increased hydrogen-ion concentration, it was found, in general, that the bending of the roots was more pronounced at pH 4.6 than at pH 7, and that the delay of root growth was also greater in the more acid solution. If the hypothesis of BLUM (1) is correct, the phototropic orientation involves two important components, the photo-reaction and the dark reaction. The first, of course, varies with the concentration of dye and with the intensity of incident light; the second should vary with the concentration of dye and with the hydrogen-ion concentration. Optimum conditions for phototropic bending must depend upon the proper selection of conditions with respect to these factors, and also, apparently, with the age and normal rate of growth of the root. It is difficult, therefore, to make generalizations with regard to the rôle of the various factors involved. For instance, our observations indicate in general that the phototropic bending is more pronounced at pH 4.6 than at pH 7.0. With strong light intensities this may be reversed, however, owing apparently to the delay of growth in the more acid solution to such an extent that the phototropic curvature is not observable.

### Active radiation

Numerous investigators have made approximate determinations of the wave lengths of radiation active in bringing about photodynamic effects (BLUM 3). All such determinations indicate that these effects are depen-

dent upon a photochemical reaction in which the dye molecule is activated by light. The following experiments have been conducted to determine the wave lengths active in inducing phototropic bending in wheat roots sensitized with erythrosin.

In these experiments a 500-watt Mazda lamp of the projection type was employed as a source, and various Corning glass filters were used to restrict the wave lengths incident on the photosensitized roots. The energy incident upon the roots was varied by placing them at different distances from the source, the reciprocal of the square of the maximum distance at which orientation of the roots in the direction of the source could be determined being taken as an index of the threshold intensity.

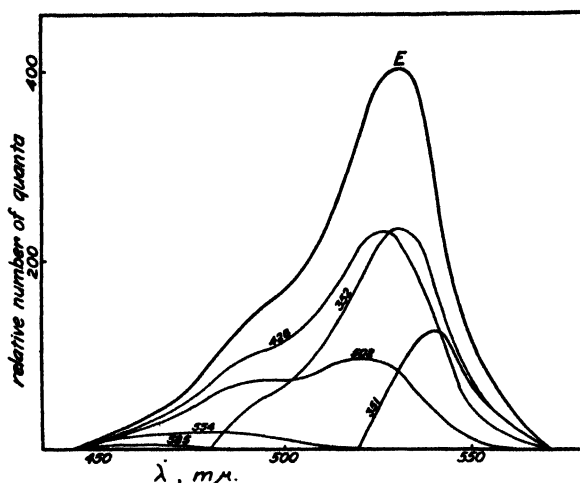


FIG. 4. Absorption curves of dye (E) and filters used.

It was found that no orientation could be obtained when the light was passed through Corning filter 348, which absorbs all radiation in the visible below 570  $m\mu$ , or through Corning filter 986, which absorbs between approximately 400 and 700  $m\mu$ . Thus the wave lengths active in producing orientation lie between 400 and 567  $m\mu$ . The erythrosin solution which was used in the experiments absorbs in the visible between 445 and 570  $m\mu$ , so that these experiments indicate at once that the active wave lengths lie within the absorption spectrum of the dye.

A still more definite correspondence between the active radiation and the absorption of the dye is shown by an analysis of the data for filters transmitting in the region of absorption of the dye. It is obvious that the true threshold intensity should be that quantity of incident radiant energy which is absorbed by the photosensitizer in the roots. This quantity of energy was estimated in the following way. The transmittance of the dye

solution used (1/500,000 erythrosin) was determined for various wave lengths by means of a spectrophotometer. According to BEER's law for absorption in solutions

$$-\log T_{\lambda} = lc K_{\lambda} \quad (1)$$

where  $T_{\lambda}$  is the transmittance or fraction of the incident light of wave length  $\lambda$  which is transmitted,  $l$  the thickness and  $c$  the concentration of the absorbing solution, and  $K_{\lambda}$  a constant for the particular wave length  $\lambda$ . Assuming that the sensitized region in the plant root represents a given thickness of dye solution of a given concentration,  $l$  and  $c$  may be taken as constant, so that

$$-\log T_{\lambda} = K'_{\lambda} \quad (2)$$

Thus  $-\log T_{\lambda}$  may be taken as an index of the relative absorption of light of wave length  $\lambda$  by the dye in the sensitized region of the root, regardless of the actual concentration of dye or the thickness of this region. The values of  $-\log T_{\lambda}$  must be corrected for the emission of the source, and when filters are used, for the transmission of the filter. If  $L$  represents the corrected values, we may write

$$L = I_{\lambda} S_{\lambda} (-\log T_{\lambda}) \quad (3)$$

where  $I_{\lambda}$  is the energy emitted by the source and  $S_{\lambda}$  the transmittance of the filter at this wave length. When no filter is used  $S_{\lambda}$  is unity, of course; and when a filter is used whose transmission is outside the absorption of the dye,  $S_{\lambda}$  is 0.

Relative values of  $I_{\lambda}$  for various wave lengths were obtained by calculating from WIEN's equation (HARRISON 5) the emission of a black body at 3280° K. This latter value is the maximum color temperature for a 500-watt Mazda projection lamp, the type used as a source. The values of  $S_{\lambda}$  for the various filters used were determined by the spectrophotometer.

Further corrections are necessary for a rigid treatment which, however, do not greatly alter the magnitude of the threshold values obtained. Actually we are not interested in the incident energy but in the relative number of light quanta incident.

If  $N_{\lambda}$  is the number of quanta for intensity  $I_{\lambda}$

$$N_{\lambda} = \frac{I_{\lambda}}{h\nu} \quad (4)$$

and since  $\nu = \frac{c}{\lambda}$

$$N'_{\lambda} = \frac{I_{\lambda}}{h\frac{c}{\lambda}} \quad (5)$$

where  $\nu$  is the frequency,  $c$  the velocity of light, and  $h$  is PLANCK'S constant.

Since  $h$  and  $c$  are constant, we may write

$$N'_\lambda = \lambda I_\lambda \quad (6)$$

where  $N'_\lambda$  is the relative number of quanta.

Correcting according to (3) we obtain

$$N'_\lambda = \lambda I_\lambda S_\lambda (-\log T_\lambda) \quad (7)$$

Under the conditions of our experiment the light must traverse a given thickness of dye solution before reaching the root. The absorption in this layer is not, of course, the same for the different wave lengths. In our experiments the thickness of solution between the root and the source was not exactly the same in all cases, but may be assumed as 1 cm. since the roots were suspended as near the center of tubes 2 cm. in diameter as was reasonably possible. To correct for this factor the values of  $N'_\lambda$  were all multiplied by the transmittance of 1 cm. of the dye solution. Since  $T_\lambda$  was calculated for 1 cm. of solution, the equation corrected for this factor becomes

$$N'_\lambda = \lambda I_\lambda S_\lambda T_\lambda \quad (8)$$

This latter correction may amount to 30 per cent. of the light quanta at certain wave lengths.

In figure 4,  $E$  represents the absorption curve of the dye corrected as above, and curves for the various Corning glass filters used are each indicated by the number of the filter. The areas under the various curves were obtained by measuring with a planimeter. The ratio of the area under any curve to the area under the total absorption curve  $E$  indicates the proportion of the light absorbed after passing a given filter to the total absorption without a filter. These ratios are the values  $A$  in table I. The relative value of the radiant energy which will just produce orientation of the sensitized root, *i.e.*, the threshold value, should be

$$\frac{A}{d^2}$$

where  $d$  is the distance from the source at which orientation can just be detected.

Obviously, if our assumptions are correct, there should be a certain degree of agreement between these threshold values. This is indicated by the data in table I, where the values of  $\frac{A}{d^2}$  are certainly of the same order.

Some difficulty was incurred in selecting the distance at which orientation first occurs. Since the roots are caused to change their direction of growth by the action of the dye even in the absence of light, some roots will nearly

**TABLE I**  
(SEE TEXT FOR EXPLANATION OF SYMBOLS)

FILTER	A	$d_{\sigma}$ (METERS)	$\frac{A}{d^2} \times 10^3$	TROPISM
0	1.00	8.00	1.6	+
		8.00	1.6	0
		3.32	1.4	+
351	0.16	4.00	1.0	0
		1.52	2.0	+
554	0.045	1.50	2.0	0
		6.17	1.3	+
352	0.48	6.17	1.3	0
		0.53	2.5	+
585	0.007	0.60	1.9	0
		3.62	2.3	+
502	0.30	4.00	1.9	0
		6.20	1.6	+
428	0.60	6.50	1.4	0
		1.10		0
348	0.00	1.10		0
246	0.00	0.10		0
986	0.00	0.25		0
			Average = 1.8	

always be directed toward the source. For this reason orientation was considered to have taken place only when a majority of the roots showed a characteristic sharp bending toward the light. To indicate the good agreement of the values of  $d$  thus taken, the values have been recorded in table I for both the greatest distance at which orientation was observed and the least distance at which no orientation occurred; these values were obtained from the results of five to eight experiments. It will be seen that the deviation from the average value is within 40 per cent., which may be considered as good agreement for this type of experiment. More exact values could probably be obtained by more precise measurements, but those obtained show a sufficiently close agreement to indicate definitely that the primary reaction in the phototropic phenomenon is the activation of the dye molecule by light.

#### Question of oxygen requirement

Attempts were made to determine whether the removal of oxygen would inhibit the phototropism, since, as pointed out by BLUM (3), molecular oxygen is characteristically required in photodynamic phenomena. It was found, however, that removal of the oxygen to a low level by bubbling  $N_2$  through the solution inhibited the root growth in itself, so that fair comparison could not be made with roots grown in air. Although this question

remains without definite answer for the present, it may be fairly assumed that molecular oxygen is necessary for induced tropisms, since this has been demonstrated in numerous other photodynamic phenomena.

### Discussion

The phenomenon of photodynamically induced tropic bending in wheat roots would appear to be based upon the same type of photochemical reaction as other photodynamic effects, namely, an oxidation of cell constituents by molecular oxygen, the dye acting as a photosensitizer. The effects of this reaction are modified, apparently, by the dark reaction of the dye. The bending appears due to a differential growth on the two sides of the root, whatever the mechanism of its production. The dark reaction may play a very important part in this differential growth, since in general the total length of roots grown in the dark in the dye solutions is found to be considerably less than those grown in the absence of dye. The introduction of dye causes unoriented bending of the roots, probably due to unequal penetration and consequent unequal inhibition of growth on opposite sides of the root as suggested above. It may be that the photochemical reaction itself serves only to alter the permeability of the growing cells to the dye, thus giving an orientation to the delay of growth by the dye. This question, of course, cannot be answered by our experiments.

The bearing of the phenomenon on tropisms in non-sensitized plants is difficult to determine at present. It seems reasonable to propose, however, that phototropic bendings may be based upon photochemical reactions of the same type as the photodynamic phenomena. In such case it would be necessary to assume that a photosensitizing substance of the general type of the photodynamic dyes is present. This is not an unreasonable assumption, since various porphyrins are found among plants and such compounds generally produce photodynamic effects. Further generalization cannot be made without thorough cytological investigations.

### Summary

1. Phototropic bending in the roots of wheat seedlings induced by the action of erythrosin and light are described.
2. It is probable that the phototropic bending represents the combined effect of the photo-reaction and the dark reaction of the dye.
3. The wave lengths of light producing the phototropic bending correspond to the absorption spectrum of the dye, indicating that the dye acts as a photosensitizer.

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# NUTRIENT ELEMENTS USED BY LEAVES AND GROWTH OF APPLE TREES<sup>1</sup>

EDMUND BURKE AND H. E. MORRIS

## Introduction

Considerable study has been given to the influence of fertilizers on the chemical composition of various parts of the tree and of the fruit, but the problem as to when the tree requires its greatest food supply has received comparatively little attention. Previous work by the writers indicates that the blossoms, leaves, and early growth remove much of the plant foods stored in the tree during its dormant period and that during the early growth the tree makes its greatest demand on the nutrient elements in the soil. The purpose of the present study was to determine to what extent the new leaves and early spring growth utilize the plant foods stored in the dormant tree, and the amount taken from the soil. The samples of apple tissue used in this investigation were obtained from the McIntosh-Morello Orchard in the Bitter Root Valley, Montana.

MURNEEK (4) maintains the necessity of an abundance of nutrients at certain critical periods for apple trees, such as during full bloom and preceding the June drop. ROBERTS (5) suggests that the failure to set is due to nutritional causes rather than to a lack of pollination or fertilization. GOURLEY (1) states that twig growth practically ceases by July 1, and shows by charts that the greatest rate of growth is during the early growing period. HOWLETT (2) gives in tabulated form the amount of nitrogen removed by flowers up to full bloom, and in his discussion of the relation of water supply to first drop states: "However, the question is still undecided as to whether the fall of bloom from spurs under ordinary circumstances is not due more to a deficiency of nitrogen than to a deficiency of water." LOEW (3), after analyzing the bark of cherry trees for several seasons before and after full bloom, found that after the blooming period the bark had lost 37.16 per cent. of protein, 30.35 per cent. of fat, and 40.59 per cent. of starch.

No attempt is made to cite all the literature relating to the importance of an abundance of plant nutrients when the tree is in blossom and leafing out. Suffice it to say that the value of an abundance of plant foods in the tree and in the soil has been recognized by several investigators.

Unpublished data by the writers show that fertilizers, especially nitrogenous fertilizers, do have a decided influence on the chemical composition

<sup>1</sup> Contribution from Montana State College, Agricultural Experiment Station, Paper no. 28, Journal Series.

of the apple fruit, twigs, leaves, and fruit spurs; and that the blossoms, young leaves, and new wood growth are exceptionally rich in nitrogen, phosphorus, and potassium. In the blossoms, young leaves, and new wood the percentages of these elements were greater than at any subsequent period. As a matter of fact, there was a decrease in the percentages of these plant nutrients in the leaves from their early formation until they dropped in the autumn. The twigs contained the greatest percentages of nitrogen and phosphorus while the tree was dormant, and the least at about the time the leaves had attained their normal size. These results seem to indicate that the greatest demand on the plant nutrients in the tree and soil is made at about the time the tree is in full bloom and the leaves and early growth are very active.

### Experimentation

In the spring of 1930, two apple trees of nearly equal size and vigor, growing near each other and about 20 years old, were selected for analysis. These trees were growing on a soil low in plant food, especially nitrogen, hence the annual terminal growth had not averaged more than 3 to 4 inches. One tree, designated the dormant tree, was removed April 9, while in a dormant state and the other, designated the active tree, was removed June 11, at about the time the leaves had attained their normal size.

The material was divided into root, trunk, large limbs, small limbs, and twigs. The green weight of each division, except the roots, was determined before samples were taken for analysis. Samples of the 1926, 1927, 1928, and 1929 growth were also taken for analysis. In addition the leaves and spring (1930) growth were removed from the limbs of the active tree, weighed, and sampled.

The nitrogen, phosphorus, calcium, and magnesium contents were determined by methods adopted by the Association of Official Agricultural Chemists. The potassium content was determined by the perchloric acid method. The moisture content of each sample was determined. A sufficient quantity of roots from each tree was dug to secure representative samples, but no attempt was made to remove the entire root system. The results of all analyses are calculated on the water-free basis.

### Chemical data

Table I gives the percentages of five mineral nutrients found in the trees. It shows that in the dormant tree there is a gradual reduction in the percentages of these plant nutrients from the 1929 growth to the trunk, but that the roots are relatively rich in these nutrients. This general trend is also apparent in the rapidly growing tree. It is of interest to note that the percentages of all plant nutrients, except potassium, are higher in the

dormant tree. This indicates that a considerable amount of these nutrient elements is stored in the tree and its root system during the dormant period. Potassium is not stored in the newer wood, but is found in largest quantities in the large limbs, trunk, and roots of the dormant tree.

TABLE I

PERCENTAGE OF NITROGEN, PHOSPHORUS, POTASSIUM, CALCIUM, AND MAGNESIUM IN A DORMANT AND IN A RAPIDLY GROWING APPLE TREE  
(PERCENTAGES CALCULATED ON WATER-FREE BASIS)

MATERIAL	TREE NO. 1 (DORMANT)				
	NITROGEN	PHOSPHORUS	POTASSIUM	CALCIUM	MAGNESIUM
	%	%	%	%	%
1929 growth	0.900	0.1787	0.484	1.948	0.1455
1928 growth	0.565	0.1156	0.191	0.985	0.0860
1927 growth	0.405	0.1068	0.177	0.953	0.0740
1926 growth	0.380	0.0855	0.164	0.904	0.0724
Small limbs	0.245	0.0655	0.135	0.629	0.0560
Large limbs (scaffold)	0.195	0.0409	0.147	0.534	0.0411
Trunk	0.120	0.0293	0.098	0.285	0.0355
Roots	0.450	0.1579	0.394	0.655	0.0842

TREE NO. 2 (ACTIVE)					
Leaves and 1930 growth	1.800	0.3720	1.840	1.419	0.2741
1929 growth	0.425	0.1263	0.490	1.583	0.0678
1928 growth	0.335	0.1008	0.351	1.124	0.0508
1927 growth	0.250	0.0957	0.299	0.955	0.0531
1926 growth	0.250	0.0831	0.245	0.975	0.0557
Small limbs	0.195	0.0813	0.219	0.883	0.0548
Large limbs (scaffold)	0.130	0.0534	0.059	0.471	0.0415
Trunk	0.095	0.0464	0.043	0.219	0.0183
Roots	0.175	0.1212	0.039	0.537	0.0688

The storage of potassium during dormancy differed from that of other nutrients examined, in that the percentages were considerably lower in the 1926 to 1929 growth, inclusive, in the dormant tree than in the rapidly growing tree. The analyses indicate that the percentages of potassium present in the different parts of the tree probably are influenced largely by sap flow. As the sap recedes most of the potassium finds its way to the roots

and lower portions of the tree, where it remains until the sap again rises. Insoluble compounds of potassium are rare and it is reasonable to assume that most of the potassium is in solution and that its distribution is governed by the flow of the sap.

Nitrogen and phosphorus differ from potassium in that they form many insoluble organic compounds. It is therefore reasonable to suppose that during the autumn months they migrate from the leaves to the new wood where they form insoluble compounds and remain during the dormant period. A portion of these elements, however, does find its way to the older wood and even to the roots.

Calcium behaves somewhat like potassium. With the exception of the 1929 growth, the percentages of calcium are higher in the upper portion of the active tree than in the dormant tree, which indicates an upward movement of the calcium in the tree when it became active.

Magnesium behaves more nearly like nitrogen and phosphorus in its distribution during the flow of sap, and it probably forms insoluble compounds which are stored in the younger growth during the dormant period.

Assuming that the two trees are alike, it is apparent from the data presented that the leaves, blossoms, and new growth make a tremendous demand on the plant food stored in a dormant tree.

TABLE II

WEIGHT OF NITROGEN, PHOSPHORUS, POTASSIUM, CALCIUM, AND MAGNESIUM IN DIFFERENT PARTS OF TREES NO. 1 AND NO. 2. TREE 1 (DORMANT) SAMPLED APRIL 9, 1930;  
TREE 2 (ACTIVE) SAMPLED JUNE 11, 1930

PARTS	TREE NO. 1 (DORMANT)					
	DRY WEIGHT	NITRO- GEN	PHOS- PHORUS	POTAS- SIUM	CAL- CIUM	MAGNE- SIUM
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Twigs and small limbs	17,288	87.99	19.54	39.24	192.93	15.39
Large limbs (scaffold)	11,088	21.62	4.53	16.30	59.21	4.56
Trunk	5,532	6.64	1.60	5.42	15.77	1.94
Total	33,908	116.25	25.67	60.96	267.91	21.89

TREE NO. 2 (ACTIVE)						
Leaves and 1930 growth	5,625	101.25	20.93	103.50	79.76	15.41
Twigs and small limbs	15,470	45.64	15.01	49.66	169.09	8.66
Large limbs (scaffold)	10,853	14.11	5.75	6.40	51.12	4.56
Trunk	4,703	4.47	2.16	2.02	10.30	0.85
Total	36,651	165.47	43.85	161.58	310.27	29.48

The total weights in grams of the five nutrients in the trees, exclusive of the roots, are given in table II. The active tree was somewhat smaller than the dormant tree, and even if the percentages of plant nutrients had been as great the total weight would be less. The data given in table III are a recalculation of the data given for the active tree in table II, assuming that, exclusive of 1930 growth, it was equal in weight to the dormant tree and that the amount of plant nutrients was increased in the same ratio.

According to the data given in table III, there were 116.25 gm. of nitro-

TABLE III

WEIGHT OF NITROGEN, PHOSPHORUS, POTASSIUM, CALCIUM, AND MAGNESIUM WHEN  
TREE NO. 1 AND TREE NO. 2 ARE CALCULATED TO EQUAL WEIGHTS

PARTS	TREE NO. 1 (DORMANT)					
	DRY WEIGHT	NITRO- GEN	PHOS- PHORUS	POTAS- SIUM	CAL- CIUM	MAGNE- SIUM
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Twigs and small limbs	17,288	87.99	19.54	39.24	192.93	15.39
Large limbs (scaffold)	11,088	21.62	4.53	16.30	59.21	4.56
Trunk	5,532	6.64	1.60	5.42	15.77	1.94
Total	33,908	116.25	25.67	60.96	267.91	21.89

TREE NO. 2 (ACTIVE)						
Leaves and 1930 growth	6,147	110.65	22.87	113.10	87.16	16.84
Twigs and small limbs	17,288	60.00	16.77	55.49	188.96	9.68
Large limbs (scaffold)	11,088	14.41	5.88	6.21	52.22	4.66
Trunk	5,532	5.26	2.54	2.38	12.12	1.00
Total in twigs, small limbs, large limbs, and trunk	33,908	79.67	25.19	64.08	253.30	15.34
Grand total	40,055	190.32	48.06	177.18	340.46	32.18

gen in the dormant tree and 79.67 gm. of nitrogen in the active tree, exclusive of leaves and 1930 growth. This means that the leaves and 1930 growth have drawn on the reserve nitrogen supply to the extent of 36.58 gm., but as the leaves and 1930 growth contained 110.65 gm. of nitrogen, the additional supply of 74.07 gm. was derived from the roots and soil.

The total weight of phosphorus in the dormant tree was 25.67 gm., while that in the active tree, exclusive of leaves and 1930 growth, was 25.19 gm., a loss of less than 0.5 gm. The leaves and 1930 growth contained 22.87 gm., however, an excess which must have come from the roots and soil.

The weight of potassium was 60.96 gm. in the dormant tree and 64.08 gm. in the active tree, exclusive of the 1930 growth. The 1930 growth and leaves contained 113.10 gm., making a total of 177.18 gm. of potassium in the active tree. This means that 116.18 gm. had to be taken from the roots and soil. It is evident from the analyses given in table I that a considerable portion of that amount was taken from the roots, for the percentage of potassium in the roots of the dormant tree was ten times the percentage found in the roots of the active tree.

The weight of calcium found in the dormant tree was 267.91 gm., while that found in the active tree, exclusive of leaves and 1930 growth, was 253.30 gm. This means that the active tree, exclusive of leaves and 1930 growth, had lost 14.61 gm. while the leaves and 1930 growth had taken up 87.16 gm. From this calculation the leaves and 1930 growth took 72.55 gm. of calcium from the roots and soil.

The weight of magnesium in the dormant tree was 21.89 gm. against 15.34 gm. in the active tree, exclusive of leaves and 1930 growth. The loss in the active tree was 6.55 gm. This means that the leaves and 1930 growth derived 10.29 gm. of magnesium from the roots and soil.

### Discussion

In considering the data presented it must be remembered that they represent the analyses of only one set of trees, and it is doubtful whether the analyses of another set would check these data in every detail; but any differences would probably not affect the interpretations. The percentages of nitrogen, phosphorus, and potassium in the 1929 growth of the dormant tree (table I) were comparable with those found in twigs collected from other orchards in the same fruit section where apple trees were making a greater growth and producing normal crops. However, the nitrogen in the leaves and 1930 growth collected on the same day from adjacent orchards contained from 2.12 to 2.25 per cent. Leaves from trees fertilized with 2 pounds of ammonium sulphate and 2 pounds of sodium nitrate per tree, in the spring of 1929 and in 1930, contained as much as 2.57 per cent. of nitrogen. These data indicate that the amount of nitrogen in the active tree and in the soil was not sufficient for normal growth. The lack of plant nutrients was further indicated by the fact that this tree blossomed heavily but failed to set fruit.

The phosphorus content of the leaves and 1930 growth of the active tree was somewhat greater than the content of comparative samples taken from unfertilized trees in another orchard, and from trees which had received a liberal application of phosphate fertilizer in the springs of 1929 and of 1930. This indicates that the phosphorus supply was sufficient for normal growth and fruiting.

The potassium content in the leaves and 1930 growth of the active tree was somewhat lower (1.84 per cent. as compared with 1.80 to 2.25 per cent.) than that found in ten samples from other trees which were making normal growth and fruiting. The leaves and new growth make a heavy potassium demand on the tree and soil, yet it is doubtful whether this percentage was sufficiently low to affect the fruiting or to interfere with the photosynthesis of the leaves.

### Summary

1. Knowledge regarding the nutrient requirements of a tree, especially during early spring, is of great importance in conducting fertilizer experiments.

2. The results of this investigation showed that the leaves and new growth of an apple tree secured from the roots and soil 66.9 per cent. of their nitrogen, 97.9 per cent. of their phosphorus, 100.0 per cent. of their potassium, 83.2 per cent. of their calcium, and 61.1 per cent. of their magnesium.

3. The active tree made a large demand on the nitrogen supply, reducing the amount stored in all parts of the tree.

4. Nitrogen stimulates tree growth more than either phosphorus or potassium, and when it is found in the soil in an available form in sufficient quantities the tree makes a normal growth and the leaves are large and dark green in color. The leaves on the active tree were small and light green in color, which confirms the belief that a lack of nitrogen was responsible for subnormal growth and failure to set fruit.

5. The phosphorus demand decreased the supply in the 1926 to 1929 growth and in the roots, while the supply in the large and small limbs increased. Comparative data indicate an ample supply of phosphorus for normal growth.

6. The percentage of potassium increased in all parts of the tree above the small limbs, but showed a decided decrease in the large limbs, trunk, and roots. The supply of potassium in various parts of the tree is largely influenced by the sap flow.

7. Calcium behaved similarly to nitrogen, and magnesium was similar to potassium in its movement.

8. A tree making rapid growth in the spring cannot depend entirely upon the nutrients stored within it, but must secure a portion of its supply from the soil.

9. It is important that soils in which trees are grown contain an ample supply of nutrient elements and that the soil be sufficiently moist to keep them available for early spring growth.

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# CONTROLLED THERMOSTATS FOR PHYSIOLOGICAL STUDIES AT LOW, NON-FREEZING TEMPERATURES

F. C. STEWARD

(WITH TWO FIGURES)

The frequent necessity for temperature control comparable with that commonly practiced in physico-chemical investigations is now somewhat generally recognized by plant physiologists. When merely a single, arbitrarily chosen temperature is adequate, the use of the now familiar gas or electrically heated ovens or thermostats which operate above room temperature offers no difficulty. In some respects, however, the physiological interest in temperatures between 0° C. and room temperature is greater than that in temperatures of the higher ranges. Especially at low temperatures it is unsafe to presume that a physiological process will have a constant temperature coefficient irrespective of the range chosen. For many purposes, therefore, it is desirable to carry out physiological determinations simultaneously at several selected, controlled, low temperatures. Not all laboratories have at their disposal a series of such control rooms, and even where these are available it is not always practicable to adjust them with the precision often required, or to repeat in separate chambers other equipment incidental to the study in question.

The arrangement here described consists of three relatively large water baths which may be maintained continuously at almost any desired temperature greater than 0° C.<sup>1</sup> The design of the baths offers the maximum amount of free space, and their size is adequate for relatively large-scale operations to be conducted in duplicate at each temperature if desired. The total cost is not excessive, and for a moderate outlay provides all the advantages of a large refrigerator cabinet plus three chambers whose temperatures may be accurately controlled at will. The whole arrangement, although compact, is somewhat more extensive than commonly assembled on the laboratory scale.

## Cooling unit and cabinet

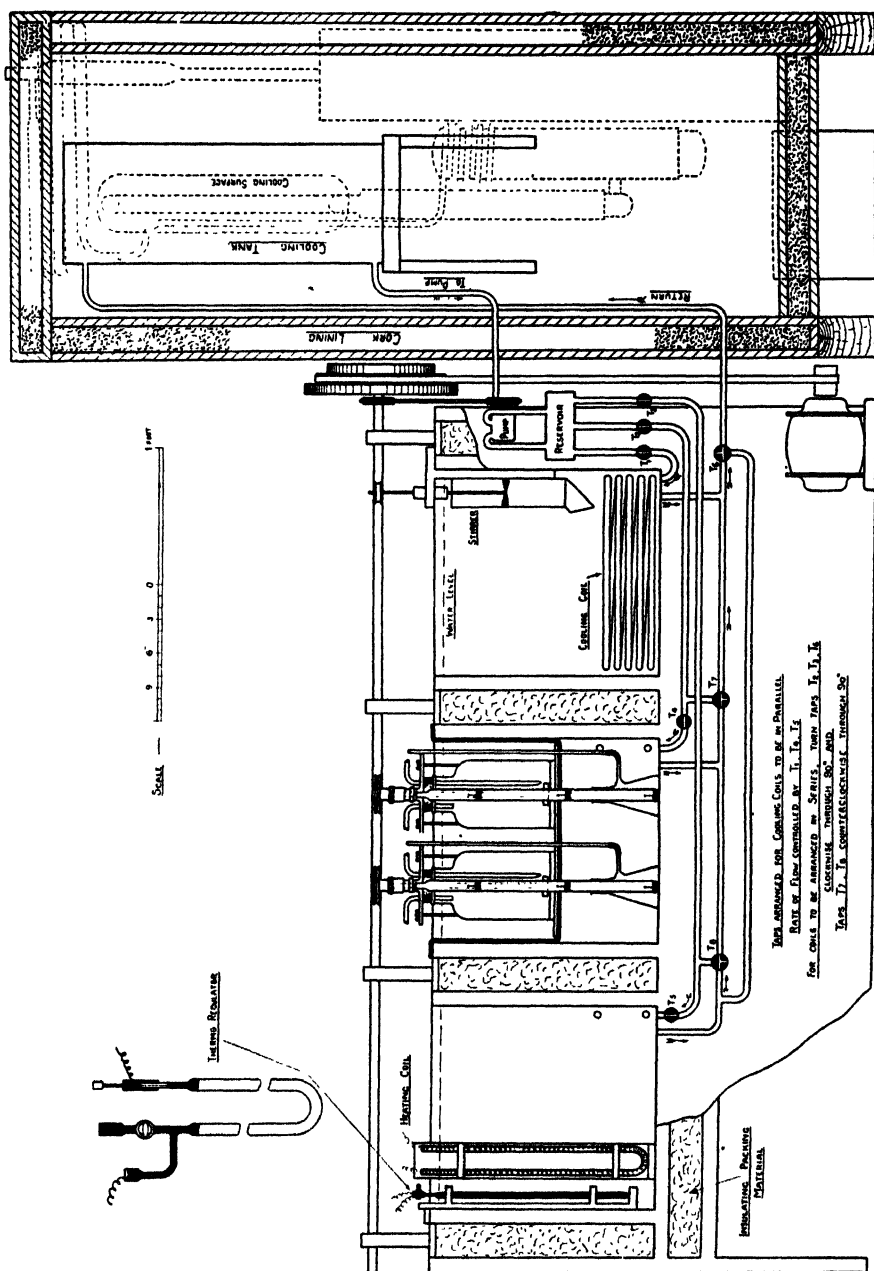
The recent advances in domestic refrigeration have developed a variety of standard refrigerators which have met with extensive laboratory use. The outlay for the larger finished models, including both unit and cabinet, is usually considerable, and they are not altogether adaptable for experimental purposes. The refrigeration units can be separately purchased at

<sup>1</sup> If necessary, the output of the unit would be adequate to use for at least one bath below 0° C.

much lower cost, however, and incorporated with laboratory apparatus. In the arrangement to be described an Electrolux unit was utilized. This model contains no moving parts, is noiseless in operation, and involves the minimum of installation as it requires only access to the mains and fixed feed and waste pipes for condenser water. The maximum output available from the model used (series 20 unit) is 1200 B.Th.U. per hour (302 Kg. cal.). This is somewhat larger than can be obtained from single units of the domestic type, although there is no reason to suppose that two smaller units operating side by side would not be equally satisfactory. This, however, might involve greater difficulties in mounting.

The unit described normally operates in a vertical position with its cylindrical cooling surface in air, but for the purpose in question it is more suitably immersed in fluid. This can be effected by building around the cooling surface a stout galvanized iron tank ( $10 \times 13 \times 26$  inches). To facilitate this arrangement, a rectangular plate may be welded<sup>2</sup> to the horizontal 2-inch pipes (technically known as the "gas heat exchange") connecting with the cooling surface, and to this the tank can be rigidly bolted, all joints being packed with red lead to prevent leakage. In this way the cooling surface can be immersed in any desired fluid, about 150 liters of which are required to cover it. The unit, which weighs some 370 lb. (117 Kg.), is securely bolted to a rigid framework having adequate supports for the cooling tank. It is necessary to reduce radiation loss from the tank to a minimum. To this end, and also to provide much of the convenience of a standard refrigerator, the cooling tank is surrounded by a large, double-walled, insulated cabinet, leaving the boiler, condenser coils, etc., outside at the rear. The space between inner and outer wall (2 in.) is completely filled with sheet refrigerator cork of this thickness. For the walls of the cabinet, horizontally arranged tongued and grooved boards ( $\frac{3}{8}$  in. thick) are adequate. In an improvised structure of this kind hinged doors are likely to cause leaks; tightly fitting, "hatch" doors (double-walled and cork-insulated), which can be removed entirely when desired, are much more satisfactory. The cabinet illustrated in the diagram (fig. 1) has one such in the lower front center ( $36'' \times 3''$ ); one in the top ( $12'' \times 19''$ ) allows access to the cooling tank; and a third (in the upper right side) is available for general access to the cabinet and is useful when it is necessary to remove accumulated frost from the sides of the tank. The total internal volume of the cabinet is 16 cu. ft.; of this about 2 cu. ft. are occupied by the cooling tank itself and the remainder (of which about 9 cu. ft. are present as a single unobstructed chamber beneath the cooling tank) is available for general cold storage purposes. Experience has shown that this

<sup>2</sup> This is best done by the manufacturer before the unit is charged with hydrogen and ammonia.



arrangement, constructed by relatively unskilled labor, is effective and has all the convenience of a much more elaborate and expensive cabinet, especially if excessive external demands are not made upon the output of the unit. In the arrangement illustrated in figure 1 no great precision was demanded when using the cabinet, but there is no reason why thermostatic control should not be incorporated if desired. An even larger cabinet could be built around the unit if adequate insulation could be provided.

### Circulating system

The cooling surface, immersed in fluid, offers a source of cold which may be distributed at will. Of the two solutions commonly used for refrigeration purposes, calcium chloride and glycerin, the latter is more satisfactory. It appears to be less corrosive to the copper cooling coils, and the viscosity of a 40:60 glycerin-water mixture by volume is not excessive. The maximum efficiency of the unit is obtained at an extraction temperature of  $-5^{\circ}\text{C}$ ., and the concentration stated is more than adequate to prevent freezing at temperatures below this. The cold glycerin-water mixture, drawn off from the base of the cooling tank, is circulated through copper coils (internal diameter 0.25", external diameter 0.375") situated at the base of each of the three thermostats. Figure 1, first compartment, illustrates this point. (To avoid overcrowding the diagram, the features of the three baths are shown in separate compartments in figure 1, although actually the fittings were replicated in all three baths. Also the circulating system has been shown diagrammatically and not exactly as *in situ*.) Each coil has four complete turns and the inlet and outlet are securely fixed to the teak wall with suitable unions. Leads, shown diagrammatically in figure 1, are arranged so that the cooling fluid traverses the three coils separately (coils in parallel) and passes by a common return to the top of the cooling tank. In this way, by controlling the rate of flow (using taps  $T_1$ ,  $T_4$ ,  $T_5$ ) to each of the three coils, the baths can be maintained at different temperatures. Experience shows that much greater control can be exercised and the desired temperatures more readily obtained if the flow through the coils can be changed at will from parallel to series. In the latter case the cooling liquid passes in turn through all three cooling coils at the same rate and three different temperatures are maintained in the baths. For this method the total rate of flow is controlled by tap  $T_1$  ( $T_2$ ,  $T_3$  are closed) and the two-way taps ( $T_6$ ,  $T_7$ ,  $T_8$ ) are adjusted as indicated in the lower part of figure 1. Since radiation from the external copper leads is considerable, all connections and metal portions must be insulated, leaving only the control taps free. A double layer of tightly wrapped thick felt provides a convenient and effective insulation. To maintain an adequate circulation of the cooling mixture, a small gear pump, such as is used for oil circu-

lation in an automobile engine, is inserted in the cooling circuit. The pump actually in use is the normal fitting for an Austin car, but this has been fitted with a gland around the driving spindle to prevent leakage of the glycerin mixture. The pump is driven by means of a chain and sprockets from the main drive of the apparatus. Two speeds are obtained by using two sizes of sprockets on the pump spindle. Since the efficiency of the cooling system depends upon a uniform rate of flow of the glycerin mixture, the chain drive described is preferable to a belt drive. When using the coils in parallel, the total flow is of the order of a pint a minute, but when using the coils in series it is considerably smaller.

### Thermostats

The three thermostats, each of which has a volume of about 5 cu. ft., are constructed of stout teak 1.25" thick, jointed and the joints sealed with red lead, and have a 3-inch space packed with insulating material surrounding them on two sides and the base (figs. 1, 2). It proved more con-

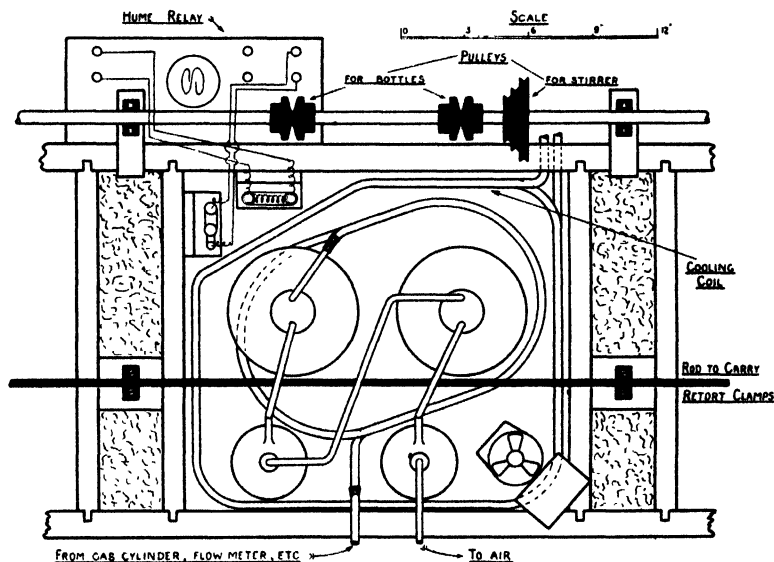


FIG. 2. Plan of one bath. Drawn by N. WARR.

venient to eliminate insulation along the length of the apparatus, as this was required for fixtures (taps, pipes, relay boxes, etc.). The three-vaned stirrers shown pull surface water down a cylindrical chimney, made of sheet zinc, and project it along the base of the bath to the heater and thermo-regulator situated diagonally opposite. The stirrer shafts are mounted on ball bearings and operate by endless leather drives from a common shaft ( $\frac{1}{8}$ " diameter, mounted on four self-adjusting ball bearings) which revolves

at approximately 200 R.P.M. Each bath is supplied with a heater and thermo-regulator circuit. By slightly overcooling the baths the heaters operate intermittently, and more constant control of temperature is obtained than if the constancy of output of the unit and rate of flow in the coils were the sole regulating mechanism. By this means the temperature variation in the baths does not markedly exceed the sensitivity of the heater and regulator circuit. Thermostatic devices are common and familiar. The one adopted has been described by HUME (1). It has been used with success in other work (2), and is simple and inexpensive, operating directly from A.C. mains. The heaters used were also of the type described by HUME. A long compact U-tube mercury thermo-regulator with one fixed and one adjustable platinum contact was fitted to each bath (fig. 1). It is a simple matter to vary the volume of mercury in the regulator and thus control the temperature at which the relay operates.

Thus far the thermostat fittings, etc., are such that the baths could be adapted to almost any physiological investigation involving various temperatures. With but little alteration the water baths could be converted into air chambers. This actual equipment, however, was intended for use in respiration and salt absorption studies analogous to those described elsewhere (3). A technique for this work under controlled conditions has already been described (2), but this did not include the use of more than one temperature in any one experiment, and this of necessity was greater than room temperature. In fact the large bath there shown has in conjunction with the apparatus here described frequently formed a fourth and higher temperature. Two of the 4-liter containers previously described (2), complete with aeration device, stirrer, and gas-tight mechanical bearings, can be accommodated in each compartment. Figure 1 (second compartment) and figure 2 illustrate the baths complete with two containers for the respiring material, modified Reiset towers for carbon-dioxide determination, and a second copper coil through which the flowing gas for aeration passes before it enters the first of the experimental vessels.<sup>3</sup> The mechanical stirrers for each respiration vessel are also operated from the main shaft by pulleys made in two sections, whose mutual adjustment controls the gear ratio for this drive.

### Working directions

It is unnecessary to give detailed working directions. A fairly constant room temperature (15°–18° C.) standardizes the losses due to radiation and increases the stability of the whole system. It is inadvisable to insert the full load suddenly, but more satisfactory to cool down the three compart-

<sup>3</sup> For further details of the devices used in regulating the flow and composition of gas, see (2).

ments one by one to the desired temperature, and then to adjust the flow of the glycerin mixture to the minimum amount necessary to maintain it (with only intermittent use of the heaters), before inserting another bath in the circuit. In this way a low cooling tank temperature is preserved and the unit exerts its maximum output. All possible necessary adjustments (rate and direction of flow of glycerin, stirring, relays, etc.) are under the control of the operator. As approximate criteria of the efficiency of the unit, the temperature of the issuing condenser water, the temperature at the base of the brine tank (which rises when the unit is overloaded), and the presence of ice on the cooling tank and on the external portions of the unit (which indicates a reserve of output) are adequate. When the external circuit is eliminated the three-step control fitted to this unit may be adjusted to maintain a low cabinet temperature (22° F.) without excessive current consumption (500 W).

By using this apparatus it has been possible to carry out efficiently and reliably, over relatively long periods of time, experiments upon respiration and salt absorption at a variety of temperatures between 0° C. and room temperature. It is suggested, however, that the utility of the system described is not confined to experiments of this nature, but that granted ordinary workshop facilities, a complete laboratory unit which embodies a large cold-storage refrigerator cabinet and three accurately controlled low temperature baths can be constructed at a moderate cost.

The writer desires to acknowledge his indebtedness to Professor J. H. PRIESTLEY, who generously provided the facilities incidental to the development of this technique. For some technical mechanical suggestions he is indebted to Mr. S. G. RICHARDSON, of the Department of Engineering, Leeds University, and for technical assistance with regard to the cooling unit to Mr. TAYLOR, of the Leeds branch of the Electrolux Company.

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ENGLAND

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## NEW METHOD OF ARRANGING EXPERIMENTS WITH VEGETABLES IN SAND CULTURES WITH FLOWING SOLUTION<sup>1</sup>

Z. ŻURBICKI

(WITH THREE FIGURES)

There is a field of research in which it is necessary to keep the contents and concentration of nutrient solutions uniform, and therefore sand cultures with flowing solutions are used. The necessary uniformity in the contents of the nutrient solution can be achieved by regulating the flow of the liquid. In the laboratories of PRIANISHNIKOFF (Agrochemical Experimental Station) and in the Agricultural Section this method was successfully used to determine the reaction of plants to different forms of nitrogen nutrition under different conditions, as well as to study the influence of different relations of the elements of the nutrient solution to the development of the plants. By such arrangement of the experiments one can be sure that during the entire period of growth the plants are feeding either with ammonia or with nitrate nitrogen. All possibility of the slightest nitrification is excluded by the rapid flow of the solution. The reaction of the medium varied within narrow limits, fixed by the measurements of pH solution flowing in and out of the vessel.

The method applied up to now, having been taken in principle from that of ALLISON and SHIVE (1), is described by DICKOUSSAR (2). Part of the arrangement remains unaltered even with the use of mechanization of the flowing sand cultures, therefore it is described briefly. The general view of the apparatus is shown in figure 1. The supply of 16 liters of nutrient solution for two days and sufficient to fill two vessels ( $B_1$  and  $B_2$ ) is placed in a bottle tightly closed with a rubber stopper. Through the stopper, the glass tube 1 and the siphon 2 are inserted. By means of siphon 2, the solution is transferred into the intermediate flask  $C$ . Atmospheric air enters the bottle  $A$  through tube 1. The solution will flow through siphon 2 from  $A$  to  $C$  until the level of the liquid of jar  $C$  reaches the height fixed by the position of the lower end of the tube 1, admitting the air. When the liquid at both ends of the siphon comes to a level the transfer of liquid stops. The solution goes from jar  $C$  through siphon 3, to vessels  $B_1$  and  $B_2$  along the two branches of siphons 4<sub>1</sub> and 4<sub>2</sub>, the speed of flow of the solution being regulated by a screw clamp or glass cock. A uniform speed of flow for the two vessels is fixed approximately, and by experience a speed is selected so that the jar will be drained in a given time. Such regulation is hard to control

<sup>1</sup> Contribution from the Central Scientific Research Institute of the Sugar Industry, Agricultural Section.

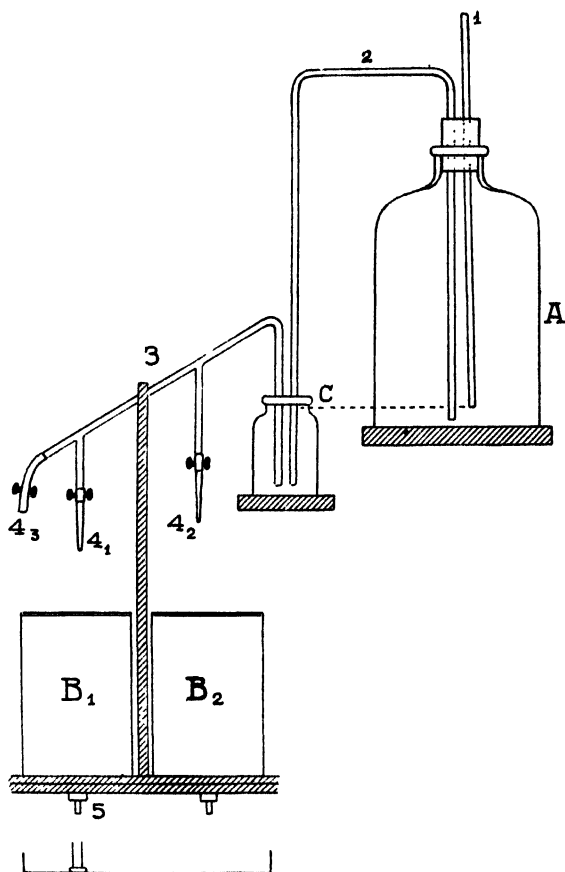


FIG. 1. Diagram of apparatus for control of flowing culture solutions.

and there is no certainty that the two vessels ( $B_1$  and  $B_2$ ) are getting the same amount of solution, and that the quantity delivered to each will be sufficient for the given time. It is especially difficult to work with the screw clamp since the rigidity of the rubber tube weakens with time, and the flow may stop altogether. The intermediate jar  $C$  maintains a steady level and creates a uniform pressure in siphon 3, or it would lower with the diminishing of the liquid in jar  $A$ , and the flow of the solution would gradually diminish.

The vessels  $B_1$  and  $B_2$  have at the bottom of each a glass tube closed by a rubber cork, with a glass tube inserted through it. To prevent the sand from entering this glass tube, it is covered with glass wool. (The solution obtained through this glass tube serves to determine the reaction.) The drops of the nutrient solution dripping from tubes  $4_1$  and  $4_2$  are falling

always at the same point in the vessel. At the same place there is a column of sand being permeated by the solution. The rest of the area of the vessel is not irrigated by the solution, and the spreading of the solution over the largest part of the vessel is greatly handicapped.

This circumstance is considered to be one of the greatest defects of this system. To start the apparatus it is necessary to force air into the tube 1 until siphon 2 is filled with liquid, then to draw the solution into siphon 3 through tube 4<sub>3</sub>, and to regulate the flow through the offshoots 4<sub>1</sub> and 4<sub>2</sub> by means of stop-cocks. During the summer of 1931, the greenhouse of the Agricultural Section modified this method in a manner that greatly facilitated the work. With the new equipment mechanized and uniform distribution of the nutrient solution in the parallel vessels ( $B_1$  and  $B_2$ ) is obtained. Within 2-3 minutes it can be calculated how long a given amount of solution will last at a given speed of flow; a thorough gravitational flow of liquid through the vessels is also obtained.

The experiment was carried out with four vessels, since work on beets with two vessels is impracticable because of the great variability of crops. All four vessels were supplied with solution from a 16-liter bottle ( $A$ ) with the same intermediate jar  $C$  as in the previous experiment. The siphon 3 was changed. Instead of the two offshoots 4<sub>1</sub> and 4<sub>2</sub> it had only one, which was introduced at the center between the two vessels.

This offshoot is connected with triplet 1 (figure 2). In the upper arm of the triplet there is a glass cock to regulate the flow of the nutrient solution simultaneously to all four vessels. The two side arms of the triplet project 2-3 cm. beyond the border of the adjacent vessel. Through the triplet the nutrient solution is divided into two equal parts for the vessels  $a$  and  $b$  on one side and for the vessels  $c$  and  $d$  on the other side. The whole triplet is made of uniform glass tubes (2 mm. in diameter) with side arms drawn out to make the outlets as uniform as possible. The exact division of the flowing liquid into two equal parts is obtained by slight turning of the support 3 in which triplet 1 is clamped. The slightest declination of the triplet from its perpendicular position is sufficient to change the amount of liquid flowing from each arm. The accurate adjustment of the triplet before use may be verified by measuring the flow of the solution. The further distribution of solution between vessels  $a$  and  $b$  on one side and  $c$  and  $d$  on the other is accomplished by the swinging curved tubes 2 fastened to a common metal support. The general arrangement of the apparatus can be seen in figure 2. The details of the swinging tubes are given in figure 3. In position 1 (figure 3) the drops of the nutrient solution are falling from the triplet into tube 1; with accumulation of the fluid in the tube the center of gravity of the whole system moves towards the position  $a$ , since the solution in tube 1 changes its position to the left of the fulcrum. At a certain

moment tube 1 tips the entire apparatus into position 2; the fluid from tube 1 is flowing into the culture vessel, while the nutrient solution is entering tube 2. After the tube has been filled with a certain amount of solution, it tips the apparatus again into position 1. Tubes 1 and 2 must both be of exactly the same glass and bent at exactly the same angle. Before the experiment is started they are both adjusted to deliver the same amount of nutrient solution. This adjustment is accomplished simply by changing



FIG. 2. Photograph of apparatus for regulating flow of culture solutions in sand.

their positions in the holders, and by finally balancing accurately with the additional weight 4 (figure 3), which works along the screw.

Before arranging the experiment the amount of fluid delivered is measured. It is difficult to make the apparatus regulate the flow exactly, but this is not imperative, since it is indifferent for the plants, whether the nutrient solution is supplied in portions of 15 or 17 cc. It is important only that both tubes deliver exactly the same amount of the solution, and distribute it equally between the vessels. The number of cc. the apparatus delivers is recorded for each apparatus.

Every now and then during the vegetative period a check of the setting should be taken. To do this it is not necessary to measure the amount of fluid delivered; it is sufficient merely to check the time necessary to fill one

of the tubes. With exact adjustment both tubes are filled in the same period of time. Since the amount of solution delivered from each tube is given, it is easy to calculate whether the contents of the supply bottle will last until the time of changing the fluid at the set speed of flow. Emptying of the tubes into the vessels is done quickly. To prevent washing out of the sand, several glass plates are introduced in such a way as to have the solution spread over half of the surface of the vessel. Each morning the vessels are turned 180° so that each half may be equally irrigated. By adding to the length and width of the bent collecting tubes, the amount of simultaneously received solution may be increased. This makes possible

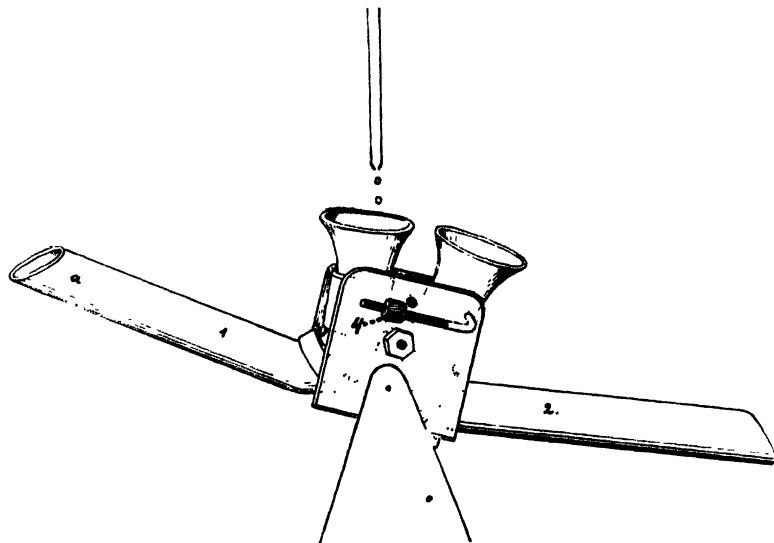


Fig. 3. Diagram showing details of swinging tubes.

a still more uniform irrigation of the entire vessel. The delivery of the solution in portions is greatly to be preferred to drip delivery, which was the old method.

The solution from the siphon is thus distributed into four parts. First the division takes place in the triplet, and second at the bottom ends of it through the swinging tubes. Opening up one cock simultaneously delivers the nutrient solution to four parallel vessels in exactly the same amounts, while before it was necessary to guess at the delivery of the solution to each vessel by regulating a screw clamp. It is not necessary to adjust the cock every day, since the triplet is always full of the solution even if the supply jar is empty. It is sufficient to replace the empty supply jar with a full one, and to add the solution to the intermediate jar to have the entire system at work again.

During the day the temperature of the solution flowing through the

tubes rises noticeably and sometimes causes accumulation of air bubbles in the triplet, which stops the outflow of the liquid. To let the air bubbles escape it is necessary only to give the cock of the triplet a full turn. It is advisable to have an extra set of supply jars, as we did in our experiments, and to prepare the nutrient solution a day before it is needed. This enables one to feed the plants continuously during the growing period.

To prevent the solution from developing algae both jars are covered with black and white paint. All the parts out of the jars are first painted with black lacquer, then with white lead. The white color prevents the solution from getting exceedingly hot. In recently published work, PIRSCHLE (3) recommends covering the surface of the vessels with procelain balls, dipped in a mixture of paraffin with coal. The black color of the balls prevents the vessel from developing algae. PIRSCHLE also rejected the previous methods of sand cultures on account of their defects, which I have pointed out previously, and adopted a system of pouring a certain amount of nutrient solution into the culture vessel by hand every 4 hours.

This method can hardly be called a successful one; besides, it calls for a night shift. It prevents the constant conditions required and introduces changing ones. The humidity in the vessels is changing, since the developing plants in 4 hours are liable during the day time to evaporate a large proportion of the water retained in the sand. The concentration of the nutrient solution is changing, and the reaction (pH) changes very greatly. These changes in reaction might not have been noted in PIRSCHLE's work if the reaction is considered to be the average pH value of the delivered nutrient solution, rather than the pH of the first portions of the solution displaced by the entrance of fresh nutrient solution.

As can be seen from our practice and bibliographical data, the methods applied up to the present do not satisfy investigators. The method suggested here for mechanization of flowing sand cultures is a great step forward in the improvement of the method itself, and also greatly facilitates the care of the experimental cultures.

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## BRIEF PAPERS

### CHARACTER OF HEMICELLULOSE IN CERTAIN FRUIT TREES<sup>1</sup>

In extension of earlier observations on the nature of hemicellulose in apple wood,<sup>2</sup> it has seemed desirable to compare the nature of this extractive in different species. Branch tissue of two seasons' growth was taken in August from apple, cherry, and pear; but in the case of peach and plum one year's growth was used. After drying at 55°, with bark attached, the material was ground to pass a 100-mesh sieve. A sample of 100 gm. from tissue freed of lipides, sugars, and starch was hydrolyzed for 3 hours with 2 liters of boiling 1.0 per cent. (wt.) H<sub>2</sub>SO<sub>4</sub>. Sugars were then separated from the neutralized extract by means of hot alcohol. The loss of weight through extraction was taken as the hemicellulose content and the result-

TABLE I  
PERCENTAGE DISTRIBUTION OF FREE SUGARS IN THE HEMICELLULOSE EXTRACT OF  
FRUIT TREE BRANCHES

SPECIES	HEMICELLULOSE IN DRY WOOD	FREE SUGARS IN HEMICELLULOSE		
		GALACTOSE	GLUCOSE	XYLOSE
Apple	20.1	4.5 +	48.2	47.3
Cherry	15.4	0.08 *	30.0	70.0
Peach	21.4	0.13 *	18.7	81.3
Pear	23.8	3.7 +	58.4	37.9
Plum	21.7	2.7 +	44.6	52.7

\* Values given were obtained by WHITTIER'S procedure (Ind. & Eng. Chem. 16: 744. 1924) following negative values derived by the Official Method; hence the + estimation for the other samples. If, as is highly probable, these values are largely due to presence of galacturonic acid, the values for xylose should be correspondingly reduced.

ing sugars were determined by conventional methods as given in BROWNE'S Handbook of Sugar Analysis. Deduction for the reducing powers of galactose and xylose gave a residual value here expressed as due to glucose, in the assembled data of table I.

The principal variations within the free sugar fraction are seen to be

<sup>1</sup> Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> Jour. Biol. Chem. 45: 407-414. 1921; Ind. & Eng. Chem. 16: 139-192. 1924.

very low contents of either galactose or galacturonic acid in the cherry and peach, together with exceptionally high proportions of xylose in these same species. It was found that the alcohol-insoluble fraction of hemicellulose in both cases contained nearly equal proportions of glucose and xylose. The results for the apple differ somewhat from those reported in the earlier examination cited, but the season of sampling and method of hydrolysis differed also.

Small samples of hemicellulose extract were prepared by the preceding methods. These were neutralized, fractionated, and subjected to determination of uronic acids by the usual decarboxylation procedure. The data obtained are shown in table II. In conjunction with the low yields of

TABLE II  
PERCENTAGES OF URONIC ACID EQUIVALENT IN THE HEMICELLULOSE OF FRUIT-TREE  
WOODS, EXPRESSED ON BASIS OF ORIGINAL DRY WOOD

SPECIES	APPLE	CHERRY	PEACH	PEAR	PLUM
1. Decomposed in hydrolysis	0.21	0.41	0.20	0.20	*
2. Free sugar fraction	1.27	0.74	0.90	0.81	1.31
3. Non-free fraction	2.07	1.26	1.54	1.95	0.74
4. Total . . .	3.55	2.41	2.64	2.96	2.05
5. No. 2 as percentage of No. 4	36	31	34	27	64

\* Negative value obtained.

mucic acid associated with the cherry and peach, as reflected in galactose values of table I, the values for these species in table II seem to suggest the occurrence of a factor other than galacturonic, such as glucuronic acid. It may be observed that the uronic acid equivalent of the free sugar fraction constituted about 30 per cent. of the total in the hemicellulose in most cases, but twice as much in the plum.

From the use of data in tables I and II, computations have been made of the proportions of both free (*i.e.*, of free sugar fraction) and total uronic acid in the hemicellulose of species here considered. The free acid ranged from about 3 to about 6 per cent., and the total from 10 to 15 per cent., of the hemicellulose. From the point of view of proportions involved, glucose and xylose would seem to be equally significant reserve components in the hemicellulose fraction. It is interesting to note that the supposed conversion of glucose to xylose had been more extensive in these samples of cherry and peach woods than in the other cases. These particular species are notable for their capacity to form pentose gums. Here, also, the transition through the intermediate hexuronic acids has been remarkably complete,

little more than traces of the latter remaining in the tissues.—W. E. TOTTINGHAM, *Department of Agricultural Chemistry, University of Wisconsin.*

## EXAMINATION OF STARCH AND HEMICELLULOSE EXTRACTS FROM APPLE WOOD<sup>1</sup>

This brief statement is presented in the hope that it may prove of service to those having occasion to determine starch and hemicellulose in woody tissues. Omitting citation of specific cases, mention may be made of prevalent dissatisfaction with the current method of expressing these fractions in terms of reducing power of their extracts. The observations were made on apple branches of one year's growth, with bark attached. Samples were ground to pass a 100-mesh sieve. After the usual extractions by ether and hot alcohol, the starch fraction was removed by use of salivary amylase. The hemicellulose fraction was extracted subsequently by boiling in 2.0 per cent.  $\text{H}_2\text{SO}_4$  for one hour.

Based upon the nearly equal reducing power of the common hexoses and pentoses, and the similarity of their anhydride factors, the reducing power of these extracts has been expressed as hexosan. In a preliminary series of samples the percentage of glucosan in the material extracted varied as follows: Sugar fraction 23.5 to 42.7, av. 30.3; starch fraction 41.9 to 88.5, av. 67.6; hemicellulose fraction 16.1 to 35.6, av. 26.9.

In another series of samples the starch and hemicellulose extracts were analyzed for contents of pentosan, uronic acid anhydride, crude protein, and ash, in addition to the usual determination of reducing power. Using the  $\text{CO}_2$  yield in distilling with 12 per cent.  $\text{HCl}$  as a measure of uronic anhydride, it has been assumed<sup>2</sup> that the latter would yield 16.7 per cent. of furfural. KROEBER's tables gave the average factor of 1.7 for converting furfural to its pentosan equivalent. From these two values was derived the factor 0.3 for converting uronic anhydride to its pentosan equivalent. The "true" pentosan content has been derived by subtracting this last equivalent from the total value of the function in question. Subtraction of the pentose equivalent of "true" pentosan from the total reducing power gave a value considered to be the true glucosan or starch. It must be recognized that the uronic acids contribute to this function but in minor degree, as a result of decomposition. There appears to be no basis, however, for evaluating this function.

In eight samples the starch extract gave recoveries as follows: ash 10 to

<sup>1</sup> Published with permission of the Director of the Wisconsin Agricultural Experiment Station.

<sup>2</sup> NANJİ, D. R., PATON, F. J., and LING, A. R. Decarboxylation of polysaccharide acids. *Jour. Soc. Chem. Ind. (Trans.)* **44**: 253T-258T. 1925.

TABLE I  
STARCH CONTENT OF APPLE WOOD, INCLUDING COMPENSATED VALUES

SAMPLE NUMBER	STARCH EXTRACT*	CRUDE STARCH†	TRUE STARCH‡	URONIC ANHYDRIDE	TRUE PENTOSAN‡	TOTAL COM- PENSATED CAR- BOHYDRATES	CRUDE PROTEIN
	%	%	%	%	%	%	%
1	5.12	2.17	1.79	1.18	0.41	3.38	0.66
2	4.00	2.12	1.44	1.16	0.68	3.28	0.41
3	8.33	4.80	3.50	2.33	1.33	7.16	0.28
4	4.52	2.10	1.22	1.72	0.81	3.75	0.42
5	10.55	5.20	3.80	1.69	1.37	6.86	0.30
6	4.50	2.70	1.89	1.67	0.96	4.32	0.70
7	13.00	8.89	8.06	1.17	0.78	10.01	1.07
8	6.83	3.22	2.60	1.37	0.61	4.58	0.73
Variability of recovery	1.0/4.2	1.0/4.2	1.0/6.6	1.0/2.0	1.0/3.3	1.0/3.1	1.0/3.8

\* Loss of weight in extraction.

† As usually determined by total reducing power.

‡ Values as compensated for overlapping determinations.

TABLE II  
HEMICELLULOSE CONTENT OF APPLE WOOD, INCLUDING COMPENSATED VALUES

SAMPLE NUMBER	HEMICEL- LULOSE EXTRACT*	CRUDE HEMICEL- LULOSE†	TRUE GLUCOSAN‡	URONIC ANHYDRIDE	TRUE PENTOSAN	TOTAL COM- PENSATED CAR- BOHYDRATES	CRUDE PROTEIN
	%	%	%	%	%	%	%
1	29.9	17.0	9.6	5.1	6.0	20.7	1.2
2	27.4	16.4	9.3	7.1	5.5	21.9	0.8
3	31.0	10.3	0.6	7.4	8.4	16.4	0.3
4	31.3	13.6	0.3	6.6	11.9	18.8	0.6
5	30.5	13.5	3.4	7.0	8.5	18.9	0.6
6	28.8	11.3	3.2	7.8	7.2	18.2	0.6
7	24.1	11.5	3.6	3.4	5.8	12.8	1.0
8	32.8	13.4	5.6	3.9	6.2	15.7	1.3
Variability of recovery	1.0/1.4	1.0/1.7	1.0/32.0	1.0/2.3	1.0/2.2	1.0/1.7	1.0/4.3

\* Loss of weight in extraction.

† As usually determined by total reducing power.

‡ Value as compensated for overlapping determinations.

26 per cent., av. 15; crude protein 5 to 26, av. 10; uronic anhydride 9 to 37, av. 23; pentosan 6 to 19, av. 12; "true" starch 27 to 62, av. 39; total determinations 89 to 150,<sup>3</sup> av. 100. The individual values have been computed to percentages of their respective samples of wood and the "true" starch compared with the usual expression for this factor. These results appear in table I, wherein successive pairs of samples represent differences in either cultural or developmental conditions which might be expected to entail a lesser starch content in the second sample. It may be seen that the "true" starch content exhibits wider ratios than the conventional starch values, in relation to tissue conditions, while the other carbohydrate constituents vary irregularly.

A similar examination of the hemicellulose extract resulted as follows: ash 9 to 17 per cent., av. 13; crude protein 1 to 4, av. 3; uronic anhydride 12 to 27, av. 21; pentosan 19 to 38, av. 25; "true" glucosan 1 to 34, av. 15; total determinations 65 to 92, av. 77. As computed to percentage of the tissue, the results are presented in table II. From these it appears that no consistent correlation exists between any or all of the carbohydrate constituents and the cultural history of the tissue. Furthermore, the "true" glucosan content varied independently of the "true" starch content. In these respects the hemicellulose fraction seems to be less definitely and directly related to plant performance than is the starch fraction.

It is recognized that the element of assumption is retained in the present treatment. Moreover, the results with one sample indicate that the compensations here applied are not generally applicable without either modification or reservation. Nevertheless the data appear to be more informative than the conventional values. From the quantitative aspect, starch was decidedly more prominent than the uronic acid constituents extracted with it; and the accompanying quantities of pentoses were rather insignificant. In the hemicellulose extract, on the other hand, the lead in proportion of extractives rotated among the constituents in question.—HENRY OTTERSON AND W. E. TOTTINGHAM, *Department of Agricultural Chemistry, University of Wisconsin.*

## DETECTION AND ESTIMATION OF FORMALDEHYDE WITHIN THE CELL OF A GREEN PLANT BY THE ALLISON APPARATUS

(WITH ONE FIGURE)

Most theories consider formaldehyde the first or at least an early step in the formation of sugars from carbon dioxide and water by the green

<sup>3</sup> Only one sample approached this departure, the next highest recovery being 114 per cent.

plant. The demonstration and estimation of this compound within the plant cell is therefore of great importance.

The inadequacy of the usual chemical methods is well presented by SPOEHR<sup>1</sup> and until now no satisfactory method has been found. The work of KLEIN and WERNER<sup>2</sup> and of POLLACCI and BERGAMASCHI<sup>3</sup> with dimedon appeared to give proof of the presence of formaldehyde in photosynthesizing plant cells. BARTON-WRIGHT and PRATT<sup>4</sup> have shown, however, that formaldomedon is formed when a solution of sodium bicarbonate is exposed to light in the absence of green plants.

Important factors that must be considered in a test to detect formaldehyde in photosynthesizing plant cells are: 1. Is the test specific? 2. Is it sufficiently sensitive? 3. Is the substance detected present in the normal cell and not, as may happen in macerated tissue, a decomposition product?

The ALLISON<sup>5, 6</sup> apparatus furnishes such a test. It gives readings specific for each compound in solution regardless of others that may be present. Compounds are detected without change. The requirements are that the solution must be sufficiently clear and uncolored to transmit adequate light to make the necessary observations, and that the concentration of the substance being detected is greater than three parts in  $10^{12}$ . Detailed descriptions of the method<sup>7</sup> may be found in the literature, so that only enough will be given here to explain the present experiment.

The solution to be investigated is placed in a glass tube through which light passes, and if minima are obtained at scale readings characteristic of a specific compound, this compound is known to be present. If solutions are of sufficient dilution, quantitative determinations may be made with the aid of a circle so adjusted as to give the number of degrees through which the Nicol prism of the apparatus must be turned just to allow or just to prevent the appearance of the desired minima.

Since considerable light can pass through small unicellular algae, such as *Chlorella*, this type of organism appeared to be a means whereby the

<sup>1</sup> SPOEHR, H. A. Photosynthesis. pp. 289-291. Chemical Catalog Co. New York. 1926.

<sup>2</sup> KLEIN, G., and WERNER, O. Formaldehyde as an intermediate product in carbon dioxide assimilation. *Biochem. Zeitschr.* **168**: 361-386. 1926.

<sup>3</sup> POLLACCI, G., and BERGAMASCHI, M. Demonstration with dimethylhydroresorcinol of formaldehyde in living plants during chlorophyll photosynthesis. *Atti Accad. Lincei* **10**: 687-689. 1929.

<sup>4</sup> BARTON-WRIGHT, E. C., and PRATT, M. C. Studies in photosynthesis. I. The formaldehyde hypothesis. *Biochem. Jour.* **24**: 1210-1216. 1930.

<sup>5</sup> ALLISON, FRED. Magneto-optic method of analysis as a new research tool. *Ind. & Eng. Chem. Analytical Ed.* **4**: 9-12. 1932.

<sup>6</sup> ———, and MURPHY, EDGAR. A magneto-optic method of analysis. *Jour. Amer. Chem. Soc.* **52**: 3796-3806. 1930.

<sup>7</sup> *Loc. cit.*

presence of formaldehyde could be detected within the plant cell. The low light requirement of such organisms for photosynthesis also makes them suitable, since the amount of light coming through the apparatus is so small that it is doubtful whether it would be sufficient for most plants to begin or to continue this process.

In testing the algal<sup>8</sup> culture for formaldehyde, a tube containing distilled water was first inserted and observations made to determine the presence of formaldehyde. The test gave negative results. A drop of the algal suspension was then introduced and the observations repeated. Minima previously determined to be those of formaldehyde appeared (scale readings 21.83 and 21.92). The contents of the tube were then filtered through hardened filter paper into another tube and the observations repeated. The minima characteristic of formaldehyde were absent, showing that the formaldehyde present when the previous readings were made was within the algal cell. To preclude the possibility that the formaldehyde was adsorbed by the filter paper from the external solution and therefore only appeared to have been in the cells, a formaldehyde solution of about the same concentration as that found in the tube with the algae was made. This solution was filtered through the same type of filter paper and was found to contain the same amount of formaldehyde before and after filtration.

A brief study was made of the rate of formaldehyde formation as affected by the time of exposure to light. The algal cells to be studied were kept in the dark over night and until placed in the apparatus for observations the next morning. Since a small amount of light must pass through the substance examined, and the amount of light necessary for photosynthesis for such algae is small, an initial negative reading could hardly be expected. The first circle reading for the appearance of formaldehyde was 3.5°, corresponding to a concentration, considering the contents of the tube as a whole, of about 3.6 parts in 10<sup>12</sup>.<sup>9</sup> This is about as small an amount as can be detected by this method. The tube and contents were then placed 2 feet from a 60-watt Mazda lamp for four periods of 7 minutes each and a final exposure of 15 minutes. Determinations were made after each exposure to determine the amount of formaldehyde present. The tube and contents were again placed in the dark and observations made the following morning. Ten-minute intervals of exposure were made. The first exposure was made 1 foot from a 25-watt Mazda lamp. The other exposures were made 2 feet from the same lamp as was used the preceding day. The results are shown in figure 1. Circle readings (increased readings show an

<sup>8</sup> Probably a species of *Chlorella*.

<sup>9</sup> This assumes that the angle corresponds to the same concentration for formaldehyde as for calcium. Details for quantitative determinations with the circle will be published later.

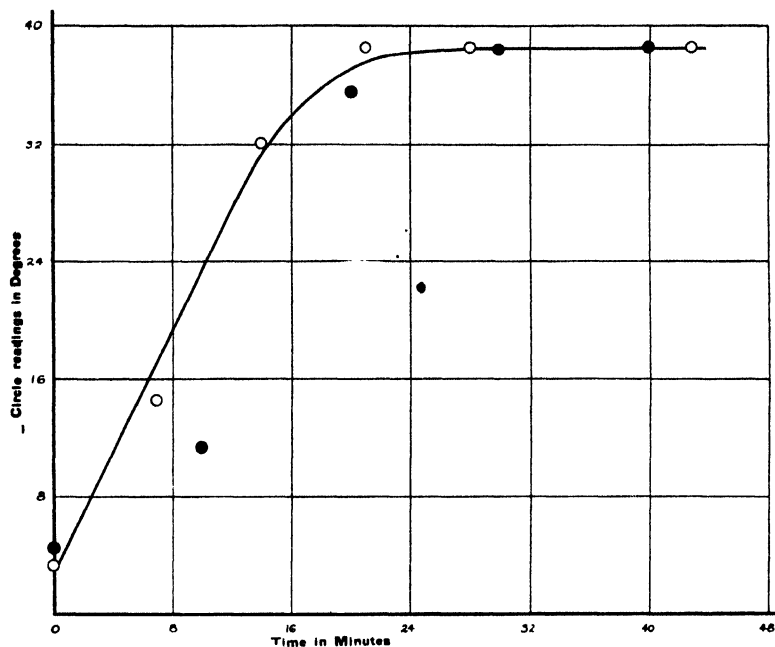


FIG. 1. Change in formaldehyde concentration with time on exposure to light: open circles, readings first day; solid circles, readings second day.

increase in concentration) were plotted against time of exposure. The final circle readings,  $38.5^{\circ}$ , correspond to a concentration of about 5 parts in  $10^{10}$ . The algae were filtered out after the final observation and no formaldehyde was then found in the solution. The concentration within the algal cell could be determined only if the ratio of the amount of algae to the water in the tube were known and providing the effect were the same as if the formaldehyde were in the solution surrounding the algae. The fact remains, however, that up to a certain time the amount of formaldehyde in the cell increases on exposure to light of a certain intensity and after that remains the same, at least for the period observed.

This preliminary work suggests great possibilities, not only in further photosynthetic studies, but in determining various metabolic products of small organisms sufficiently transparent to allow enough light to pass through for work with the ALLISON apparatus. The minima for comparatively few organic compounds have been determined and the task of finding them may be long and tedious. Once these minima are found, the presence of compounds in solution in organisms of suitable types, such as bacteria and small, comparatively transparent fungi and algae, can easily be detected.—ANNA L. SOMMER, EDNA R. BISHOP, AND IRENE G. OTTO, *Alabama Agricultural Experiment Station, Auburn, Alabama.*



## NOTES

**Tenth Annual Meeting.**—The tenth annual meeting of the American Society of Plant Physiologists will be held in Boston, Massachusetts, on December 28–30, 1933. The hotel headquarters is the Hotel Statler, Boston. The banquet will be held on Thursday evening at the Brunswick Hotel, Cambridge. A business meeting has been tentatively arranged for the afternoon of December 29. A large attendance is desirable at the business meeting, since it is constitutionally possible to transact business with final decisions, if the attendance is sufficiently large, without resorting to balloting by mail. As the banquet is the main social function of the meeting, members will find it desirable to arrive as early as possible, and to procure banquet tickets immediately on the opening day.

**Program Committee.**—The program committee for the Boston meeting consists of the following members: Dr. CARL G. DEUBER, Yale University; Dr. H. F. BERGMAN, United States Department of Agriculture; Dr. T. G. PHILLIPS, University of New Hampshire; Dr. DOROTHY DAY, Smith College; and Dr. B. E. GILBERT, Rhode Island Agricultural Experiment Station, chairman of the committee. The secretary is *ex officio* a member of the committee. Plans have been tentatively arranged for an attractive meeting which no one would wish to miss.

**Membership Committee.**—The membership committee, under the leadership of Dr. LAURENZ GREENE, of Purdue University, has been conducting an active campaign to hold and increase the membership of the Society. Other members of the committee are Dr. F. P. CULLINAN, U. S. Department of Agriculture; Dr. A. R. DAVIS, University of California; Dr. G. T. NIGHTINGALE, New Jersey Agricultural Experiment Station; and Dr. C. F. KORSTIAN, Duke University. The cooperation of all members in this work is highly desirable. If two new members were enlisted in each of the 48 states, the membership would reach a new high record. Special efforts to maintain and increase library support is desirable, also, since some of the more meagerly supported libraries found it necessary to discontinue subscriptions temporarily. Any member whose file is not complete as to available volumes can assist by purchasing back numbers as far as they are still obtainable. The first two volumes of PLANT PHYSIOLOGY have been exhausted for several years, and volumes three and four are scarce. The last four volumes are held in sufficient number to supply any probable demand for them in the near future. In order to main-

tain an uncongested outlet for publication of research in plant physiology, we should have support sufficient to publish about 1000 pages annually instead of 600.

**Barnes Life Membership Committee.**—The Barnes Life Membership committee has been appointed by Dr. C. O. APPLEMAN, president of the Society, and the selection of the candidate for the award will be made under the liberalized by-laws which were adopted at the time of the annual election last June. The committee consists of five members, as follows: Dr. WALTER F. LOEHWING, University of Iowa; Dr. S. V. EATON, University of Chicago; Dr. CHARLES E. SANDO, U. S. Department of Agriculture; Dr. GEORGE P. BURNS, University of Vermont; and Dr. T. G. PHILLIPS, University of New Hampshire, chairman. The selection is usually announced at the annual banquet, an event which adds much pleasure to that function.

**Stephen Hales Address.**—The third STEPHEN HALES address will be presented before the Society at the Boston Meeting by Dr. HUBERT BRADFORD VICKERY, to whom was awarded for his excellent work on vegetable proteins the third STEPHEN HALES prize at the ninth annual meeting at Atlantic City in 1932.

**Early Collection of Dues.**—Since it is necessary to use our funds for publication conservatively and with caution, the editors of PLANT PHYSIOLOGY will appreciate an early response to the secretary's notices of dues for 1934. The constitution makes October 1 the date at which the dues for the following calendar year become collectible. This provision was made to enable the secretary to make up a relatively complete subscription list by January 1, so that no loss might be incurred by sending the January number of the journal to those who find it necessary to discontinue membership. Early payment of dues gives the editors opportunity to plan the scale of publication for the succeeding calendar year with intelligence and with confidence in the continuity of support.

**Jethro Tull.**—This year, 1933, is the 200th anniversary of the publication of JETHRO TULL's great book, *Horse-Hoeing Husbandry*, which was published in Dublin by A. RHAMES in 1733. The memorial committee of the Society has decided to include a brief memorial program at the tenth annual meeting in honor of this event.

**Wound Compensation, Transplantation, and Chimaeras in Plants.**—The press of Julius Springer, Berlin, has published an extensive mono-

graph by N. P. KRENKE entitled *Wundkompensation Transplantation und Chimären bei Pflanzen*, in a German edition, with N. BUSCH and O. MORITZ, of Kiel, as translator and editor respectively. It is no. 29 of the *Monographien aus der Gesamtgebiet der Physiologie der Pflanzen und der Tiere*. The first section of the volume deals with natural mechanical factors, and the second part with artificial (chirurgical) factors in the production of abnormal growths, transplantation, and chimaeras. The first section is relatively brief (pp. 1-138), the second part extensive (pp. 139-877). This second section considers such topics as the reaction of cells and tissues to wounding; wound compensation; the results of transplantations; and the phenomena of growth chimaeras. The final section and appendix touch upon the problems of immunity, internal therapy, and serological relations of grafted tissues to one another. A great deal of information has been brought together in this interesting field. It is a valuable source book for investigators of such problems. The prices at which the publisher quotes it are RM 88 in brochure, and RM 89.8 in cloth binding. Orders may be placed directly with the publishers.

**Handbook of Plant Analysis.**—The fourth and final volume of this extensive handbook by G. KLEIN has been issued in two volumes paged consecutively. The two volumes together have 1868 pages. There is a table of constants for known plant constituents (pp. 1441-1706), which gives the melting point, boiling point, solubility, and optical rotations as far as they are known.

The first half of volume IV takes up methods for determining amino acids, amides, amines, proteins, purine and pyrimidine compounds, nuclein bodies, alkaloids, cerebrosides, and some unclassified constituents. The second half takes up methods of enzyme determination, methods of studying fermentation (carbohydrases, esterases, proteases, nucleases, amidases, and desmolases), antigens and antibodies, plant hormones, and vitamins. Some special methods of biological analysis are included, such as water examination, soil analysis, fermentation media, nitrogen fractionation, fractionation and preparation of pure substances by chromatographic adsorption.

The four volumes of this work bring together the methods for all kinds of chemical investigations of plant materials. Those who attempt to use the work should not forget that in compiling these methods it has not been possible to consider them very critically. The field covered has been too broad for any one individual or even for the entire group of authors involved to do more than state the methods, without critical comments as to the degree of accuracy and as to the precautions which must be taken

in their use. Such critical studies will devolve upon the users of the work.

The publisher is Julius Springer, whose Vienna plant has been issuing this set. The prices quoted for the two halves of vol. IV are RM 190 for brochure, and RM 198 for cloth bound copies. Address Julius Springer, Schottengasse 4, Wien I, Austria.

**Errata.**—Members and subscribers are requested to note errata for volumes 7 and 8 at the close of the table of contents in this number.

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